Buffering noise: KAT2A modular contributions to stabilization of transcription and cell identity in cancer and development

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Highlights

- KAT2A maintains cellular phenotypes by limiting transcriptional variability
- KAT2A-containing complexes SAGA and ATAC control distinct phenotypes
- KAT2A maintenance of leukemia stem cells is likely exerted through ATAC

Abstract

KAT2A is a histone acetyltransferase recently identified as a vulnerability in at least some forms of Acute Myeloid Leukemia (AML). Its loss or inhibition prompts leukemia stem cells out of self-renewal and into differentiation with ultimate exhaustion of the leukemia pool. We have recently linked the Kat2a requirement in AML to control of transcriptional noise, reflecting an evolutionary-conserved role of Kat2a in promoting burst-like promoter activity and stabilizing gene expression. We suggest that through this role, Kat2a contributes to preservation of cell identity. KAT2A exerts its acetyltransferase activity in the context of two macromolecular complexes, Spt-Ada-Gcn5-Acetyltransferase (SAGA) and Ada-Two-A-Containing (ATAC), but the specific contribution of each complex to stabilization of gene expression is currently unknown. By reviewing specific gene targets and requirements of the two complexes in cancer and development, we suggest that SAGA regulates lineage-specific programs, and ATAC maintains biosynthetic activity through control of ribosomal protein and translation-associated genes, on which cells may be differentially dependent. While our data suggest that KAT2A-mediated regulation of transcriptional noise in AML may be exerted through ATAC, we discuss potential caveats and probe general *vs.* complex-specific contributions of KAT2A to transcriptional stability, with implications for control and perturbation of cell identity.

Correct specification of cell fate or identity is critical for tissue homeostasis and can be achieved through tight transcriptional control. KAT2A is a histone acetyltransferase that catalyzes *in vivo* deposition of acetyl groups at Histone 3 Lysine 9 (H3K9), and to a lesser extent H3K14 [1,2]. More recently, KAT2A has also been shown to mediate deposition of other acyl residues, namely succinylation of H3K79 [3]. All these modifications associate with transcription activation, and KAT2A has been suggested to stabilize, but not necessarily initiate [4], locus transcriptional activity in mammalian cells. In addition to histone acylations, KAT2A catalyzes lysine acetylation of non-histone proteins, which may have activating (e.g. Egr2 [5], E2A-PBX1[6] or repressive (e.g. Cebpα [7], PGC-1α [8]) consequences for protein activity. Some of its targets include transcription factors [7–9] indicating an additional pathway into transcriptional regulation. Other targets are effector proteins, such as p53 [10], α-tubulin [11], and cell cycle regulators including CDC6 [12], cyclin A [13] and PLK4 [14]. In recent years, Tora and colleagues used shot-gun proteomics to catalogue proteins endogenously acetylated by KAT2A [14]. Further to targets above, the global KAT2A acetylome was shown to include targets involved in phosphorylation (MAP4K4, PRKG2, STK4), actin-mediated cell contraction (MYH2, TPM3) and protein transport (RHOD, XPO1). In this Perspective, we will focus on histone acetylation roles of KAT2A, which have been more extensively characterized.

KAT2A plays crucial roles in development. Kat2a is one of 2 highly homologous orthologues of Gcn5, the first described histone acetyltransferase [15]. The other orthologue, Kat2b/Pcaf, has a largely mutually exclusive pattern of expression with Kat2a. Kat2a dominates in hematopoiesis, neural tissue, as well as in development [16], whereas Kat2b prevails in skeletal muscle. Kat2a plays essential roles in development along the evolutionary scale. It is essential for metamorphosis and oogenesis in *D. melanogaster* [17]. In the developing mouse embryo, Kat2a is ubiquitously expressed between E7.5 and E9.0, with the exception of heart and allantois, and its expression decreases after E16.5 [18], which could suggest reduced contribution in terminal differentiation. Conversely, Kat2b is minimally expressed at early stages of development, and upregulated in adult tissues, particularly in the heart and skeletal muscle [16,18]. Kat2a, but not Kat2b, is essential for mammalian embryonic development. *Kat2a* null mice die at E10.5, with extensive mesodermal apoptosis; embryos also display defects in notochord, somites, and in neural tube formation [19]. Despite normal development and unhindered viability of *Kat2b* null mice [19], double *Kat2a/Kat2b* mutants [19] have a more severe phenotype than single *Kat2a* null animals, and

die earlier at E7.5, suggesting functional redundancy. Redundancy between the 2 paralogs has also been proposed in zebrafish, where combined perturbation of *Kat2a* and *Kat2b* results in more severe heart and fin developmental defects than single gene loss [20].

Kat2a is not strictly required for maintenance of pluripotent mouse embryonic stem cells (ESCs) [21], but it stabilizes pluripotency gene regulatory networks [22] and allows progression of reprogramming in induced pluripotent stem (iPS) cells [19]. Additionally, Kat2a is required for cell survival and correct lineage specification and differentiation in embryoid bodies [21], specifically through regulation of FGF signaling [23]. Kat2a acts as a co-factor to Myc family proteins, namely c- and N-Myc, both of which play key roles in embryogenesis [24] as well as in maintenance of pluripotency [25]. Kat2a acetylates Myc, promoting its stability [26], and is essential for activation of Myc target genes through histone acetylation [27]. Kat2a is also required for proliferation of neural stem and progenitor cells, phenocopying the role of N-Myc [28].

KAT2A has been shown to modulate osteogenic differentiation of periodontal stem cells through inhibition of the Wnt/ ß-catenin pathway by DKK1 [2]. Specifically, KAT2A mediates H3K9/K14ac of *DKK1* promoter, activating its expression [2]. In hematopoiesis, Kat2a has been described as a requirement in lymphoid blood lineages, but, interestingly, not in blood stem cells [7,29]. In more detail, Kat2a participates in maturation of specific B [30] and T cell subtypes [31], as well as in differentiation of innate natural killer T (iNKT) cells [5]. Kat2a roles in B and T cell development involve promoter histone acetylation. On the other hand, Kat2a participates in iNKT cell differentiation through non-histone protein acetylation and activation of the transcription factor Egr2. Kat2a-mediated acetylation of Cebpa, in contrast, represses its transcriptional activity and limits progression of terminal granulocytic differentiation [32].

KAT2A contributes to cancer through control of transcriptional activity. High KAT2A expression associates with bad prognosis in Breast Cancer [33–35], Non-Small Cell Lung Carcinoma [36,37] and Colon Cancer [38] namely through histone acetylation-mediated co-activation of E2F and MYC transcriptional targets to maintain cell proliferation and survival (**Figure 1A**). In Melanoma, increased KAT2A stability by *WDHD1* leads to enhanced H3K9 and H3K56ac levels [39]. Increased expression of KAT2A also associates with poor survival in Renal Cell Carcinoma (**Figure 1B**), but the mechanism has not been elucidated. In contrast, high levels of KAT2A confer a survival advantage in Pancreatic Adenocarcinoma

(**Figure 1C**), and, to a lesser extent, in low grade Glioma (Cox coefficient = -0.167, FDR=0.125), suggesting that KAT2A may act in an oncogene-like, as well as in a tumor suppressor-like manner. Interestingly, KAT2A succinylation activity has recently been linked to tumor maintenance in Pancreatic Adenocarcinoma cell lines [40] (**Figure 1A**). This raises the possibility that KAT2A may have cancer stage-specific roles, as shown for other epigenetic regulators [41], although mechanistic data in support of this hypothesis is currently lacking. Significantly, no recurrent *KAT2A* mutations have been described in cancer, suggesting that it is co-opted by tumor cells at an epigenetic level for establishment and/or maintenance of tumorigenic programs. The lack of mutations could indeed reflect conflicting roles of *KAT2A* loss or over-expression at different stages of cancer progression, with no sustained selective advantage. This is contrast with disease-specific effects, which would more likely associate with recurrent mutations in individual tumors.

We identified KAT2A as a candidate vulnerability in AML through a CRISPR drop-out screen of AML cell lines [42], representing a subset of genetic abnormalities commonly found in the clinic. AML is a heterogeneous disease, encompassing a spectrum of biological histories and prognoses dependent on the mutational event initiating or driving the disease [43], as well as their target cell [44,45]. We showed that KAT2A acetyltransferase activity maintains undifferentiated cultured and patient-derived human AML cells *in vitro*. *In vivo*, genetic knockout of *Kat2a* was incompatible with long-term preservation of functional leukemia stem-like cells (LSCs) in the *MLL-AF9* AML model [29]. *Kat2a*-depleted (KO) LSCs lost repopulating capacity, but, interestingly, did not fully progress through myelo-monocytic differentiation. Specifically, we performed single-cell transcriptional analysis of *Kat2a* wild-type (WT) and KO leukemia and inspected relative cell composition and differentiation trajectories from LSCs to differentiated leukemia cells. WT *MLL-AF9* cells aligned along an almost linear trajectory. In contrast, *Kat2a* KO leukemia cells were distributed along multiple discontinuous differentiation trajectories. We interpreted this observation as reflecting multiple uncoordinated routes into cell fate decision-making, which were initiated but not coherently completed by cells depleted of stem cell potential. We observed a similar scenario of incoherent cell diversification upon Kat2a catalytic inhibition in mouse ESCs [22]. Indeed, Kat2a-inhibited cells were incapable of sustaining pluripotency, but lagged in their ability to proceed through lineage differentiation and remained frozen in a slowly-exiting and largely irreversible transition state limbo.

Kat2a regulates noise or variability in transcription. At a molecular level, the instability of cell state identities generated upon *Kat2a* loss corresponded to enhanced cell-to-cell variability or noise in the expression of multiple individual genes [22,29]. Transcription is inherently variable, as individual genes are transcribed episodically, rather than continuously, in what are known as transcriptional bursts [46]. Bursts are characterized by a frequency – the rate of gene promoter switch from OFF to ON status – and a size – the number of mRNA molecules transcribed in a burst. The higher the burst frequency [47], or the lower the burst size-to-burst frequency ratio [48], the lower the variability in gene transcription. Mathematically, variability can be measured as coefficient of variation, CV= standard deviation/mean. Read across multiple genes, and multiple cells, transcriptional noise or variability is captured as cell-to-cell heterogeneity in gene expression (**Figure 2**). Transcriptional variability can be advantageous and has indeed been posited to provide an adaptive background for response to environmental changes or stress conditions [49]. It has also been recurrently associated with transitions between homeostatic cell fates (reviewed in [50]. Generically, cells benefit from transcriptional variability to adapt or evolve. But cells also employ mechanisms to limit variability and ensure that functional maturation processes can progress with minimal perturbation [51]. Promoter sequence [52], multiplicity of transcription factor binding [53], and chromatin configuration [54] have been shown to participate in transcriptional variability. More directly, chromatin regulators can buffer or enhance variability [55], as one potential mechanism by which to influence cell fate transitions and ultimately, identities.

The yeast orthologue of Kat2a, Gcn5, is well recognized as a transcriptional noise buffer [56], its loss increasing locus-specific gene expression CV [55]. We captured a similar effect for Kat2a in mammalian systems [22,29]. The variability in transcription resultant from *Kat2a* loss affects a large number of genes [29], at least some of which in an uncoordinated manner [22]. As a consequence, individual gene participation in gene regulatory networks is modified, with the possibility of destruction and/or reassembly of regulatory programs, and the cell identities they configure [50,57]. The resulting molecular disarray is thus likely responsible for (1) the loss of stem cell function and (2) the inability to fully organize a differentiation pathway, as we observed in pluripotency [22] and in leukemia [29]. In other words, KAT2A functions to keep cell identity by preserving the stability of the cell underlying transcriptional programs.

KAT2A exerts its lysine acetyltransferase function in the context of 2 macromolecular complexes, but their individual contribution to transcriptional stability and cell identity control is currently underexplored.

KAT2A participates in SAGA and ATAC complexes. Both KAT2A-containing complexes, respectively Spt-Ada-Gcn5-Acetyltransferase (SAGA) and Ada-Two-A-Containing (ATAC) (**Figure 3**) are key to its efficient lysine acetylation activity [58]. SAGA is evolutionary conserved from yeast to human [59]. ATAC is a smaller complex characteristic of multicellular organisms, initially associated with chromatin functions in *Drosophila* [60,61]. Structural and functional organization of SAGA and ATAC complexes in model organisms has been extensively reviewed elsewhere [62–64]. We consider them briefly here and include information from recent structural studies of the yeast SAGA core [65,66].

Mammalian SAGA is composed of 20 subunits organised in distinct functional and structural modules. The HAT module, shared with ATAC consists of: KAT2A (or the mutually exclusive KAT2B), SGF29, TADA3 and a complex-specific TADA2 variant –TADA2B in SAGA and TADA2A in ATAC. SGF29 is a tandem TUDOR domain protein that recruits KAT2A activity to active promoters marked by H3K4 tri or di-methylation [67]. TADA3, is a transcriptional activator adaptor required for transcriptional activity [68]. TADA2 variants are zinc finger (ZF) proteins that can bind double-stranded DNA [69]. The central Core of SAGA is formed of TADA1, suppressor of Ty (SPT) elements, SUPT3H, SUPT7L and SUPT20H, and of TATA-binding protein associated factors (TAFs) - TAF5L, TAF6L, TAF9, TAF10 and TAF12 [62]. TAF5L and TAF6L are SAGA-specific, whilst the other TAFs are shared with TFIID complex, the RNA Polymerase II (PolII) General Transcription Factor. The structure of the yeast SAGA Core has been recently resolved [65,66], revealing an octamer-like fold organization consisting of Taf6l-Taf9, Taf12-Ada1 (TADA1 orthologue), and Taf10-Spt7 (SUPT7L orthologue) pairs, each contributing with a Histone-Fold (HF) domain [65,66]. Spt3 (SUPT3H orthologue) contributes with 2HF domains and assumes a free conformation key to binding of SAGA to TATA-binding protein (TBP) [66]. The histone octamer connects to the remaining Core via Taf5l [65]. SAGA includes a large transcription factor interaction module, TRRAP, originally identified as cofactor of c-Myc and E2F proteins [70]. TRRAP is also found in NuA4/TIP60 HAT complexes [71] and in the ATP-dependent chromatin remodeling p400 complex [72], with possible complex-unique as well as complex-shared or redundant functions. SAGA also contains a Splicing module composed

of SF3B3 and SF3B5, which is only observed in multicellular organisms [68,73]. SF3B3 is a component of the splicing factor SF3B essential for spliceosome assembly [74], and links SAGA to the general splicing machinery [63]. Finally, SAGA comprises a lysine de-ubiquitination (DUB) module, catalyzed by USP22, which targets H2B and H2A as well as non-histone proteins [75]. All 4 members of the DUB module – USP22, ATXN7L3, ATXN7 and ENY2 – are needed for full deubiquitinating activity [76]. ENY2 participates in another chromatin complex, TREX-2, involved in mRNA nuclear export [77]. In yeast, SAGA enzymatic modules HAT and DUB, associate with the central Core in a flexible way [65,66], which may warrant functional independence. Indeed, previous studies showed that loss of DUB components does not impair SAGA complex integrity or HAT activity [78–81]. Additionally, HAT and DUB can exist as separate entities [82,83], supporting the notion of alternative HAT and DUB SAGA-independent roles.

The second KAT2A-containing complex, ATAC, includes: YEATS2, a H3K27ac reader [84] which allows for integration of additional transcription activation signals; DR1/NC2β, also present in the NC2 complex, which heterodimerizes with YEATS2 to interact with TBP; ZZZ3, a ZF protein that specifically binds H3 tails [85]and WDR5, shared with the MLL methyltransferase complexes [86], and therefore potentially important in establishing a crosstalk between H3K4me [87] and H3K9ac chromatin marks, which complements or extends the roles of SGF29 double TUDOR domain. Furthermore, Wdr5 and Kat2a are bound by the WNT-interacting protein Pygo2, which brings together Mll2-containing and Kat2a-containing complexes to activate expression of WNT targets, including *Myc* in mammary epithelial cells [35]. Within the context of MLL complexes, WDR5 was identified as a candidate therapeutic target in CEBPA N-terminal leukemia [88] but its functions within ATAC, particularly in the context of cancer, including leukemia, remain poorly understood. In an interesting additional link, two long non-coding RNAs [89,90] were shown to promote gastric cancer through scaffolding of KAT2A and WDR5 at a subset of promoters and activation of gene expression. The remaining ATAC components are MBIP, a MAP3K regulator exclusive to mammalian cell complexes [86]; and a second HAT activity – KAT14 [91]. YEATS2, MBIP and KAT14 are required for complex integrity [91]. In mammalian cells, ATAC acetylates both H3 and H4, preferentially through KAT2A and KAT14 respective activities [63,85] whereas in *Drosophila* the complex preferentially targets H4. ATAC binds Host Cell Factor 1 (HCFC1) [86], a scaffold to multiple chromatin-modifying complexes [92], and POLE3, the DNA polymerase epsilon subunit 3 [86].

Transcriptional noise-susceptible signature in AML is ATAC-associated. SAGA and ATAC have distinct chromatin targets [67], which for the most part function in general regulatory cell processes, as we have indeed observed for Kat2a itself [22,29]. Kat2a complexes likely operate across multiple cell types at least in part through stabilization of gene expression programs that are recurrent between cells. In this context, cell specificity may be achieved by unique uses or by differential reliance of individual cell types on general regulatory programs. In principle, both Kat2a-containing complexes can make contributions to stability of transcription, and through it, to maintenance of cell identity. The noise/variability buffering role of Kat2a is conserved from yeast, in which only SAGA is present, suggesting that the role may be preserved within that complex. Indeed, in yeast, loss of Ubp8 (USP22 orthologue), also results in increased expression CV, albeit less extensively and to a lesser degree than Gcn5 [55]. However, the mechanism may be different to Gcn5, as loss of Ubp8 impacts transcription burst size, as well as burst frequency, in line with a degree of independence of SAGA catalytic modules. The same is noted of Sus1 (ENY2 orthologue) [55]. In the case of ENY2, it is possible that its participation in the nuclear export complex TREX-2 may make an additional contribution to control of variability in transcription [93], although the relative amplification of mRNA variability in the cytoplasm *vs* the nucleus remains controversial [94,95]. Of note, the HAT component Sgf29 [55], which is in common between the SAGA and ATAC HAT modules in multicellular organisms, also affects burst size and frequency. This suggests a distinct activity of Gcn5/KAT2A itself in buffering transcriptional variability, which, in multicellular organisms, could be conveyed through either complex. Indeed, integration of transcriptional activation signals, namely through ATAC YEATS2 and WDR5, as well as through the common SGF29, suggests that ATAC may participate in stability of transcription, particularly as the H3K27ac mark read by YEATS2 can increase transcriptional burst frequency [96], and reduce transcriptional variability, or noise.

Our data in the *MLL-AF9* leukemia model suggests a stronger association of variability control with the ATAC complex. Inspection of H3K9 acetylated promoters lost in *Kat2a* KO primary leukemias [29] revealed similar associations with SUPT20H (SAGA) and ZZZ3 (ATAC) bound promoters as retrieved from the ENCODE database (SAGA: Odds ratio, OR=1.43, FDR=$1.78\text{x}10^{-9}$, GM12878 data set; ATAC: OR=1.31, FDR=$2.37\text{x}10^{-2}$). In contrast, variable genes with increased expression CV and/or decreased burst frequency in response to *Kat2a* loss, were more strongly enriched in ZZZ3 targets (OR=3.28, FDR=$1.75\text{x}10^{-3}$) than in SUPT20H-bound genes (OR=1.77, FDR=$1.18\text{x}10^{-2}$), suggesting that

control of transcriptional noise, at least in the context of *MLL-AF9*-AML, may be dependent on the ATAC complex. It is possible that evolutionary diversification of KAT2A into a second complex could have transferred functions from SAGA to the newer ATAC. Indeed, yeast SAGA Ada2 is more similar to ATAC TADA2A than to SAGA TADA2B [62], supporting the notion of functional reassignment between complexes. Alternatively, KAT2A-mediated control of transcriptional noise may be independent of the complex environment, but selective in terms of the affected genes, for example as a function of co-existing chromatin modifications [54].

ATAC is a generic requirement in cancer, with SAGA displaying lineage specificity. In our analysis of mouse *MLL-AF9* leukemia, most of the genes that responded to *Kat2a* loss with increased transcriptional variability were involved in ribosomal biosynthetic and translational activity. The same classes of genes are targeted by ATAC components ZZZ3 [85] and YEATS2 [84] in Lung Carcinoma, in which both genes, as well as *KAT2A* [36] are required. Cancer cells depend on high protein production [97,98] and it is perhaps unsurprising that regulators of ribosome biogenesis constitute dependencies across multiple malignancies. Global analysis of CRISPR drop-out screens through interrogation of the Cancer Dependency Map (DepMap) Project at the Broad Institute, shows that 4 ATAC components – *TADA2A*, *YEATS2*, *WDR5* and *DR1* –, the first two of which are exclusive to ATAC, are called "essential", as they constitute genetic vulnerabilities in >75% of the cell lines studied. In contrast, SAGA-specific elements were not called "essential", suggesting more specific and potentially more targetable [99] roles in cancer. ATAC and SAGA-specific subunits had distinct lineage associations, as determined by statistical strength of dependencies across cell lines representing the same tissue. ATAC elements *ZZZ3* and *YEATS*2 have a strong statistical association with blood cells (**Figure 4A**), specifically lymphocytic malignancies, likely reflecting the association between KAT2A and lymphoid cell differentiation. Our own CRISPR screen of AML cell lines [42] did not identify dependencies on ATAC elements, which may indicate more subtle requirements in myeloid lineages. SAGA-specific elements also associate with blood malignancies of lymphocytic lineages – Non-Hodgkin Lymphoma and Multiple Myeloma but have stronger associations with tumors of the Central Nervous System and Renal Cell Carcinoma (**Figure 4B**). Indeed, they mimic *KAT2A* dependencies, suggesting that ATAC may exert KAT2A-independent effects or compensate for its loss with other HAT activities. *KAT2B* does not constitute a dependency on any cell line analyzed, while *KAT14* is required in a small number of cell

297 lines with B-lymphocyte bias, making it a more likely candidate for *KAT2A* redundancy.
298 Effects on control of transcriptional variability may nevertheless be exclusive to KAT2A,
299 allowing for more subtle effects on cancer cell maintenance that do not translate into an
300 absolute requirement.
301 In the context of SAGA, similarities extend to the DUB module, particularly *USP22* and
302 *ATXN7*, which associate preferentially with Renal Cell Carcinoma (both) and Lymphoid
303 malignancies (*ATXN7*). *ATXN7* has indeed been reported to associate with prognosis of Renal
304 Cell Carcinoma [100]. Furthermore, it confers susceptibility to Breast Cancer [101] and
305 *ATXN7* gene variants have been linked to post-operative prognosis of HBV-related
306 Hepatocellular Carcinoma [102], all of these tumors to which *KAT2A* makes oncogene-like
307 contributions [33–35,103]. *USP22* acts as an oncogene in multiple malignancies [104,105],
308 including in NSCLC [106]and in Gastric Cancer [107]. However, it also displays tumor-
309 suppressor roles, specifically in Colorectal Cancer through decreased mTOR activity [108],
310 and surprisingly, in AML [109]. In AML, USP22 deubiquitinates and stabilizes PU.1. In the
311 absence of USP22, degradation of PU.1 leads to a block in myeloid cell differentiation, which
312 promotes *K-Ras* mutant leukemia progression [109]. This is in contrast with our reported
313 oncogenic function of *Kat2a* in AML [29], and may denote the previously described degree
314 of independence between the DUB and HAT modules. Alternatively, the contrasting effects
315 of *Kat2a* and *Usp22* loss may be explained by the fact that different forms of AML were
316 analyzed in both studies. These may rely on distinct leukemia-initiating cells and be driven
317 by different mutational signatures, which may determine specific dependencies on activation
318 or repression of individual genes. Nevertheless, analysis of mouse ESCs also supports a
319 degree of independence between *Usp22* and *Kat2a*: *Usp22* is uniquely required for
320 appropriate differentiation into the 3 germ layers through repression of pluripotency master
321 regulator *Sox2* [110], a role that exceeds the *Kat2a* requirement in pluripotent cells [21,22].
322 Similarly, although *Usp22* and *Kat2a* KO mouse models display embryonic lethality at E10.5
323 [111], *Usp22* KO embryos present generalized apoptosis, which is not restricted to
324 mesodermal structures as in *Kat2a* KO [18]. ATAC *Kat14* KO mouse model [91], is also
325 embryonic lethal at E10.5, with cell cycle defects and localized apoptosis that does not
326 completely overlap with Kat2a, particularly in mesodermal structures. Again, this observation
327 is compatible with independent functions of the complexes, and within complexes, of the
328 catalytic units. Additionally, there may be compensation by Kat2b, which like Kat2a can
329 function in SAGA and ATAC complexes. SAGA Core *Supt20* hypomorphs, on the other
330 hand, are more akin to *Kat2a* hypomorphic animals [112–114] with neural tube and axial

skeleton developmental defects. Similarly, in mouse ESCs, Core components *Taf5l* and *Taf6l* maintain low levels of differentiation through activation of a MYC regulatory network [115], which is largely shared with Kat2a [19]. The loss of SAGA core elements is thus more aligned with *Kat2a*, resembling the pattern observed in lineage affiliation of cancer dependencies. In fly, however, KAT2A essential roles are closely aligned with ATAC, with its requirement in metamorphosis [17] more similar to ATAC *Ada2a* [116] than with SAGA *Ada2b* or the DUB module, both of which are dispensable at this stage [62,78].

Taken together, the examples suggest that ATAC regulates fundamental biosynthetic activities, which are conveyed through Kat2a with more or less extensive participation of Kat14. ATAC Kat14 may compensate for *Kat2a* loss, at least in some lineages. SAGA, on the other hand, performs lineage-specific functions, with minimal compensation of Kat2a activity within the complex, and with some independence of function between HAT and DUB modules. It is likely that ATAC-mediated Kat2a acetylation activity maintains self-renewal and/or survival of proliferative, metabolically active cells, while Kat2a functions within SAGA to sustain cell identity and prevent deviation from existing transcriptional programs. One implication of cell identity control through SAGA is that the genes and programs targeted by the complex are less likely to be shared between tissues and cell types. Attribution of Kat2a-mediated control of individual loci to one or other complex, requires testing of complex binding on a tissue-specific basis, as publicly available data from other cell models may not reliably represent targets in a given cell type. This is more likely to be relevant for SAGA and could for example have influenced our suggestion of ATAC-biased control of transcriptional variability by Kat2a. More extensive cataloguing of complex-specific targets in different cells, and manipulation of Kat2a locus activity with complex specificity will shed light on SAGA and ATAC distinct roles in control of transcriptional activity.

Conclusion

KAT2A is a lysine acetyltransferase that stabilizes active transcription predominantly through H3K9ac acetylation, and which we recently identified as a dependence in AML stem-like cells. In mouse leukemia driven by the *MLL-AF9* fusion, Kat2a-mediated histone lysine acetylation of gene promoters enhances the frequency of transcriptional bursting and likely maintains a stable transcriptional program that preserves the functional identity of the cell. Destabilization of transcription may transiently alter gene regulatory networks and allow

cells to access alternative states and functions that deviate from the existing *status quo*, with or without successful establishment of new identities. This phenomenon may have different consequences for different cells, depending on their differentiation status, and in the context of cancer, of their genetic composition, with the possibility of disease-specific and/or stage-specific effects. KAT2A can function as the enzymatic moiety of 2 distinct multiprotein complexes: SAGA and ATAC. ATAC contributes more closely to biosynthetic roles, including translation and protein synthesis. SAGA may regulate more cell-specific transcriptional targets, including by modulating splicing. Thus, SAGA configures a more direct role in keeping cell identity, whereas ATAC may differentially sustain cell function and survival as a measure of their dependence on protein biosynthetic activity. Although we have highlighted KAT2A's role in limiting gene transcription variability, it remains unclear whether both complexes participate in variability control, or indeed if KAT2A makes additional contributions to transcriptional activity that are complex-specific. Clarification of specific KAT2A functions within the complexes, and their respective roles in development and disease, will illuminate the contribution of KAT2A to transcriptional noise or variability buffering, and in governing cell identity and differentiation. Importantly, this information can steer safe and efficient targeting of KAT2A in cancer, including leukemia, with minimal consequences to healthy tissues.

Figure legends

Figure 1. KAT2A participates in cancer biology with oncogene-like and tumor-suppressor roles. A. Described mechanisms of participation of *KAT2A* in different malignancies. **B.** KAT2A as an oncogene: Kaplan-Meier survival curves for Renal Clear Cell Carcinoma patients with high (25%) and low (25%) levels of KAT2A expression; log-rank analysis, p-value = 8.45e-11. **C.** KAT2A as a candidate tumor suppressor: survival curves for Pancreatic Adenocarcinoma patients with high (25%) and low (25%) levels of KAT2A expression; log-rank analysis, p-value = 0.025. Data in B and C were retrieved from OncoLnc (*oncolnc.org*; as of August 2020*)*, a tool that links The Cancer Genomics Atlas (TCGA) survival data to mRNA, miRNA, or lncRNAs expression (Anaya et al 2016). Despite KAT2A requirements in individual cancers, including AML, KAT2A levels do not systematically affect patient survival raising the possibility of stage or disease-specific effects.

Figure 2. Schematic model of transcription noise or variability and associated consequences to cell identity. A. Low gene expression noise associates with high frequency of promoter activation, or bursting, which maintains relatively invariant levels of gene expression. Invariant gene expression, in turn, reduces the probability of cell fate transitions and preserves cell identity. In this case, maintained high expression of the blue gene preserves blue cell fate. **B.** High gene expression noise is largely a consequence of reduced frequency of promoter activation of bursting. This allows individual transcript levels to vary more widely over time,

sometimes resulting in gene expression configurations incompatible with maintenance of the current cell identity. This is demonstrated in this example by high expression of the purple gene, above the normally dominant blue expression, resulting in a purple cell fate. Alternatively, the expression of both genes may fall simultaneously resulting in absence of cell identity signals and eventual apoptosis.

Figure 3. Schematic representation of the human SAGA (left) and ATAC (right) KAT2A-containing complexes. SAGA (left) is organized into distinct structural and functional modules. Subunits belonging to each module are colored similarly. The KAT2A-containing histone acetyltransferase (HAT) module is depicted in orange and shared with ATAC (right), with exception of TADA2B, which is replaced by TADA2A in ATAC. The histone de-ubiquitinase (DUB) module is shown in red; the Core module in turquoise. TF-binding module, TRRAP, is shown in yellow. Splicing module is depicted in purple. TATA-binding protein (TBP), in grey, is not part of the complex architecture but it associates with SUPT3H to recruit SAGA to TATA box and facilitate transcription. Apart from the HAT domain, ATAC subunits do not have a modular organization.

Figure 4. Analysis of dependencies and lineage associations of ATAC and SAGA-unique elements in CRISPR drop-out screens of cancer cell lines. Data was extracted from the Cancer Dependency Map - DepMap (https://depmap.org/portal/) as of August 2020. Unique elements in each complex are highlighted with a black edge. **A.** ATAC elements (*TADA2A, YEATS2, ZZZ3, MBIP, KAT14*) are required in most cell lines and are called as 'common essential genes'. Upon grouping of cell lines into specific tissues, or cell lineages, lineages for which requirements of individual genes were determined at p-value<0.0005 are considered as a lineage association bias. Bars reflect the total number of cell lines in each lineage associated with the various ATAC-unique elements. **B.** SAGA unique elements (*TADA2B*, *SUPT20H, SUPT3H*, *SUPT7L*, *TAF5L*, *TAF6L*, *TADA1*, *ATXN7*, *ATXN7L3*, *USP22*) are highlighted with a black edge. None of the elements was called 'common essential'. Their lineage associations are depicted in the bar graph, as per the criteria in A.

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Conflict of interest disclosure

The authors have no conflicts of interest regarding the content of this article.

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Figure 1

A

KAT2A functions in solid tumours

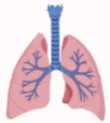
Melanoma

Stabilised by *WDHD1*

↓

↑H3K9ac and H3K56ac
[39]

Lung cancer



Interacts with E2F1

↓

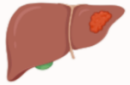
↑H3K9ac

↓

↑*E2F1, CCND1, CCNE1* expression
[36]

Increased protein levels
[37]

Hepatocellular carcinoma



Associates with E2F1

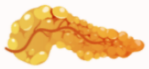
↓

↑H3K9ac of E2F1 binding site at the *AIB1* promoter

↓

↑*AIB1* expression
[97]

Pancreatic adenocarcinoma



H3K79succ

↓

↑*YWHAZ* expression

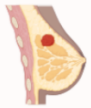
↓

↑β-catenin stability

↓

↑*CCND1, MYC, GLUT1, LDHA* expression
[40]

Breast cancer

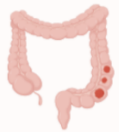


Mediates
TGF-β-1 - induced EMT
[34]

Interacts with Pygo2
↑ Wnt/β-catenin -
target gene expression
[35]

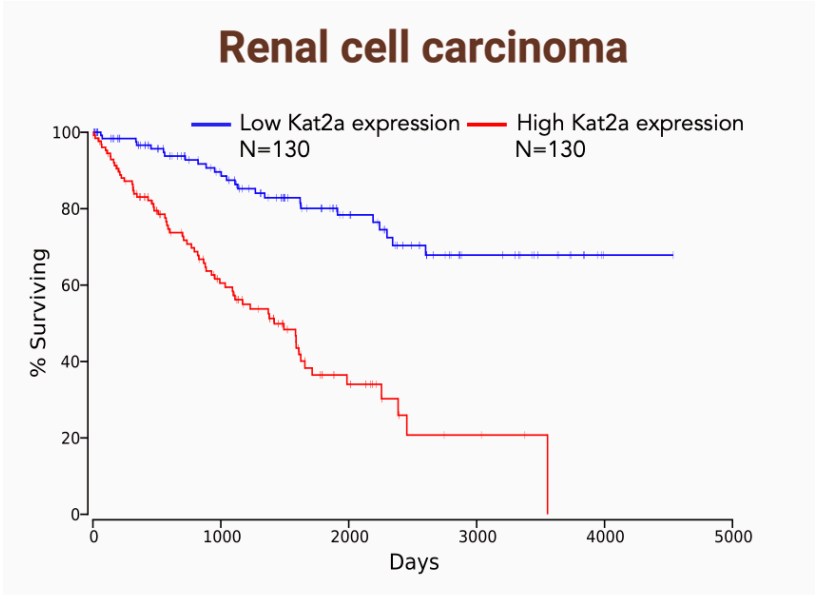
Mediates HBXIP-induced
α-tubulin acetylation
[33]

Colon cancer



Increased transcriptional
activity stimulated by E2F1
[38]

B



C

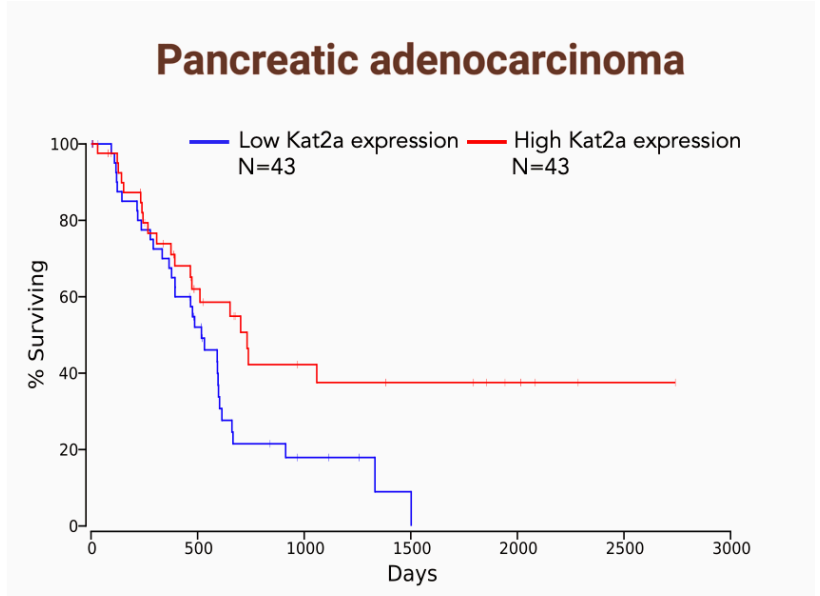
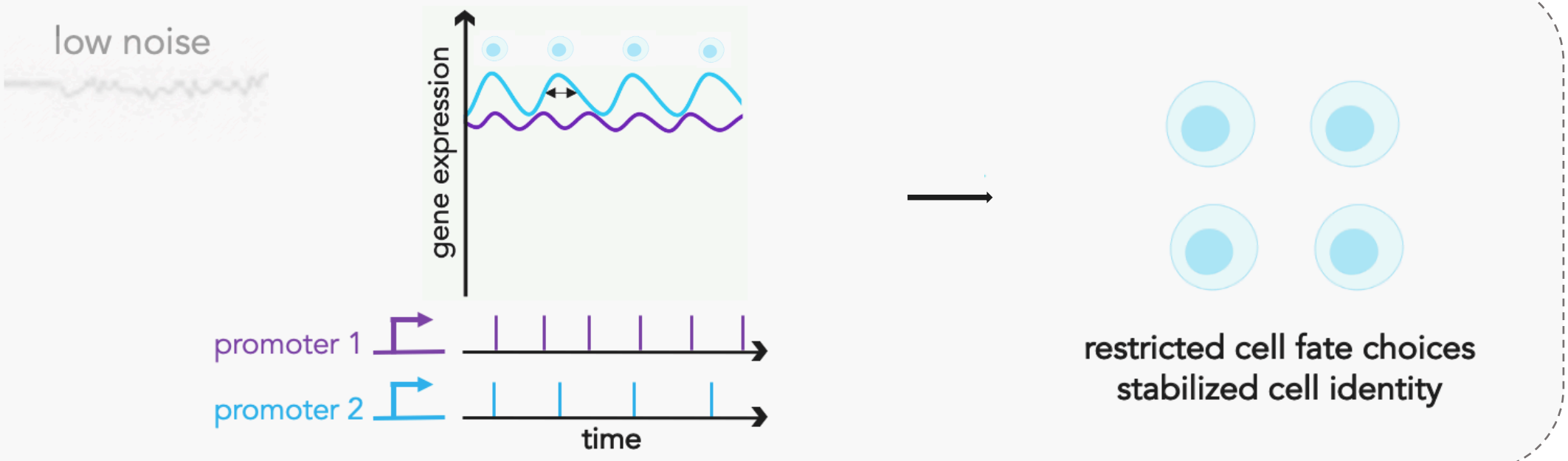


Figure 2

A


low noise
gene expression
promoter 1
promoter 2
time
restricted cell fate choices
stabilized cell identity

B

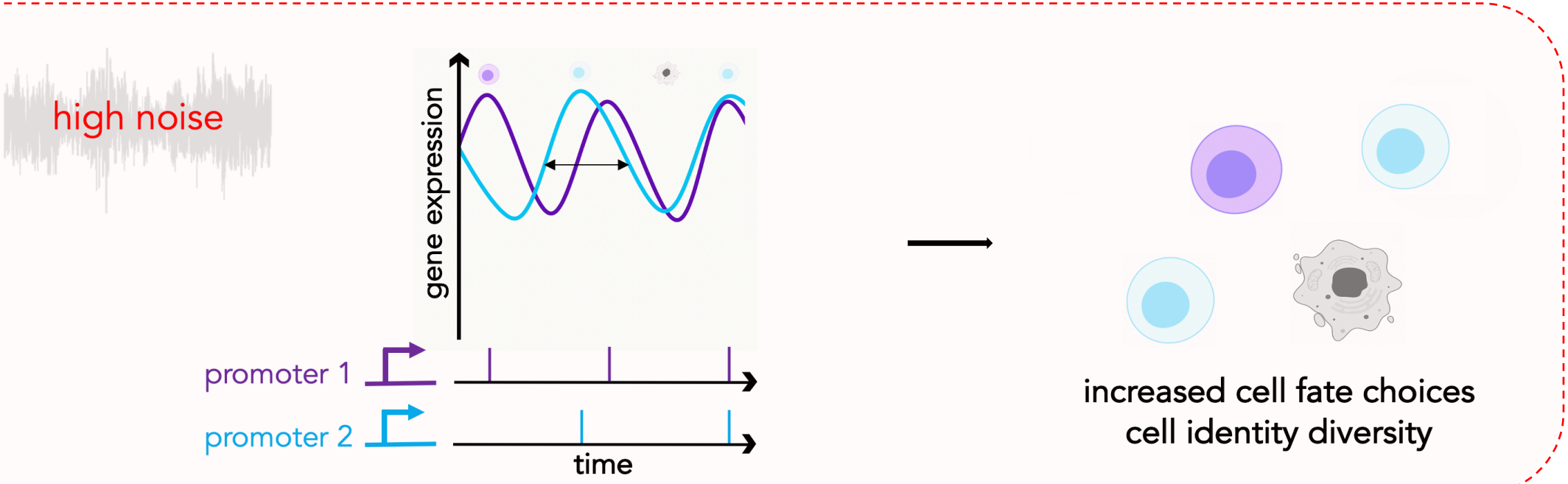

high noise
gene expression
promoter 1
promoter 2
time
increased cell fate choices
cell identity diversity

Figure 3

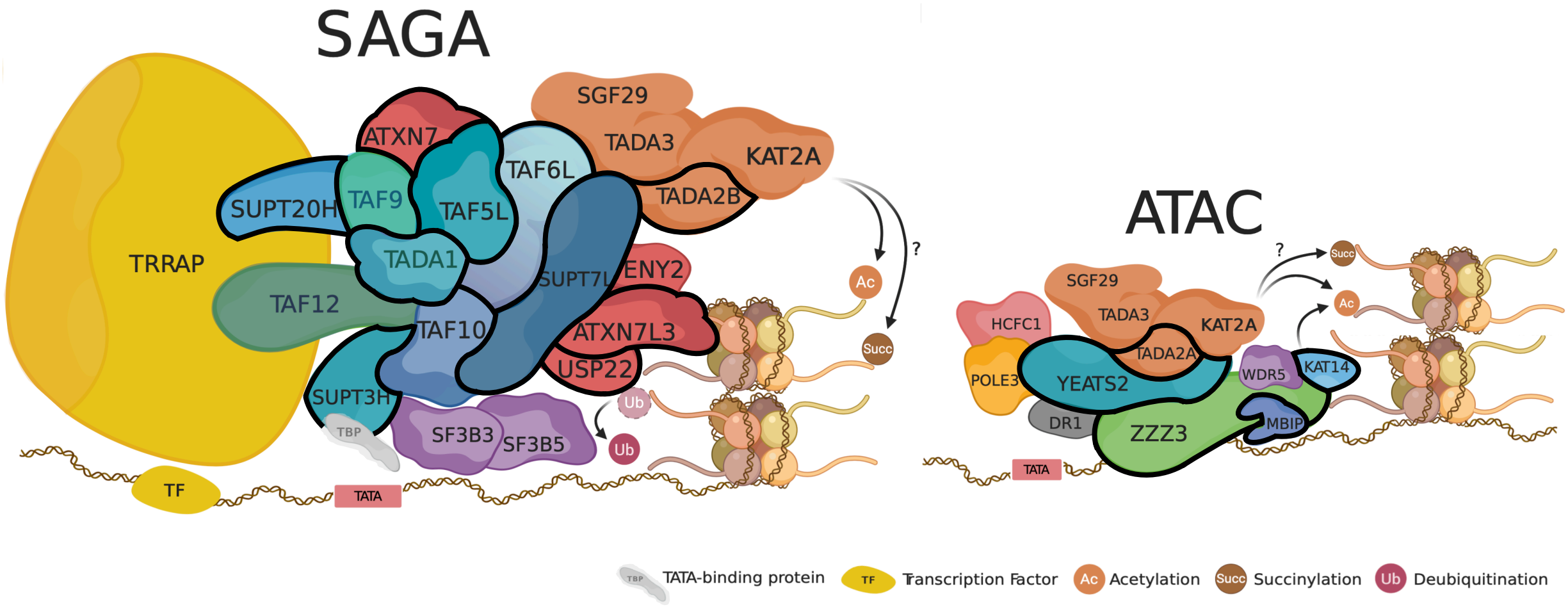
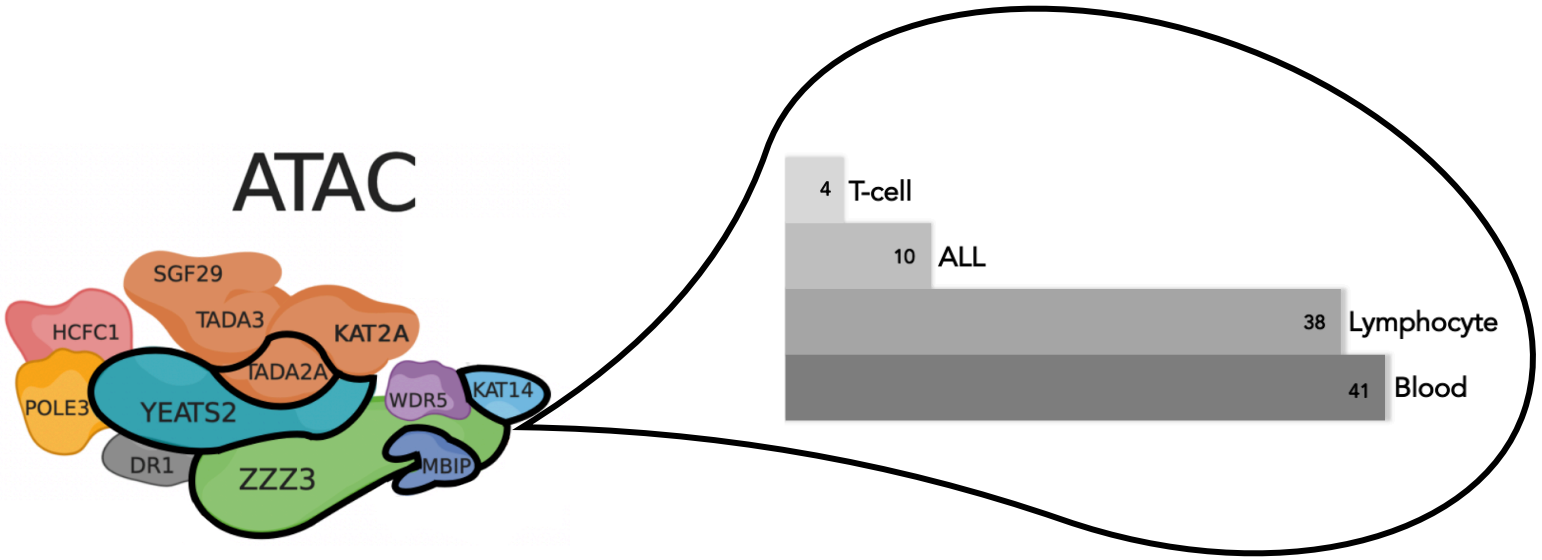

SAGA
TRRAP
ATXN7
SUPT20H
TAF9
TAF5L
TAF6L
SGF29
TADA3
KAT2A
TADA2B
TADA1
TAF12
TAF10
SUPT7L
ENY2
ATXN7L3
USP22
SUPT3H
TBP
SF3B3
SF3B5
TF
TATA
Ac
Succ
Ub
?
ATAC
SGF29
HCFC1
TADA3
KAT2A
TADA2A
POLE3
YEATS2
WDR5
KAT14
DR1
ZZZ3
MBIP
TATA
TATA-binding protein
Transcription Factor
Acetylation
Succinylation
Deubiquitination

Figure 4

A

lineage associations of ATAC-specific elements



B

lineage associations of SAGA-specific elements