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ATRX

The case of a peculiar chromatin remodeler

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The SWI/SNF-like chromatin remodeler ATRX has recently garnered renewed attention. *ATRX* mutations were first identified in patients bearing the syndrome after which it is named, alpha thalassemia/mental retardation, X-linked. While ATRX has long been implicated in transcriptional regulation through multiple mechanisms, recent studies have identified a role for ATRX in the regulation of histone variant deposition. In addition, current reports describe *ATRX* to be mutated at high percentages in multiple tumor types, suggestive of a potential "driver" role in cancer. Here we discuss the numerous and seemingly diverse roles for ATRX in transcriptional regulation and histone deposition and suggest that ATRX's effects are mediated by its regulation of histones within the chromatin template.

Keywords: ATRX, histone variants, telomeres, α-globin, macroH2A and H3.3

Abbreviations: ChIP, chromatin immunoprecipitation; TSS, transcription start site; ESC, embryonic stem cell; TR, tandem repeat; G4, G quadruplex; PTM, post-translational modification

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ATRX and its Syndrome

A study of patients presenting mental retardation, developmental delay and distinctive facial features, associated with α-thalassemia, led to the mapping of mutations in a helicase encoded on the X chromosome, named *ATRX*.[1,2] Intriguingly, ATRX syndrome mutations lie in two distinct domains: either the N-terminal ADD (ATRX-DNMT3-DNMT3L) domain,[3] which contains a GATA-like domain and a histone tail binding plant homeodomain (PHD),[4-6] or the C-terminal helicase region,[7] characteristic of ATP-dependent chromatin remodelers (**Fig. 1**).[8,9] Interestingly, these mutations often result in substantially reduced ATRX protein levels in patients.[10] However, the mechanisms by which loss of functional ATRX results in these syndrome features, including reduced α-globin expression and mental retardation, remain unclear.[11]

ATRX and Condensed Chromatin

Studies over the last ~15 years have uncovered multiple roles for ATRX, some of which may appear to be contradictory. For example, the fact that *ATRX* mutations result in the loss of α-*globin* gene expression and that ATRX physically binds to the α-*globin* gene cluster, suggests a transcriptionally activating role for ATRX.[10,12] However, its localization to telomeres, pericentric heterochromatin and the inactive X chromosome[13-15] implicate a role in the establishment and/or maintenance of transcriptionally silent chromatin. The protein interaction partners of ATRX, including HP1α, EZH2, MeCP2 and macroH2A, also implicate its role in heterochromatin structure and function (**Fig. 1**).[16-20] Additionally, the N-terminal ADD domain of ATRX, which binds the heterochromatin-associated histone modification H3K9me3 (see below), bears homology to domains present in DNA methyltransferases,[3,21-23] also suggesting transcriptional repression. Mechanistic insight into ATRX function is presently emerging and the picture is not so clear-cut—ATRX appears to have many faces in regards to its role in histone deposition and transcription, as discussed below.

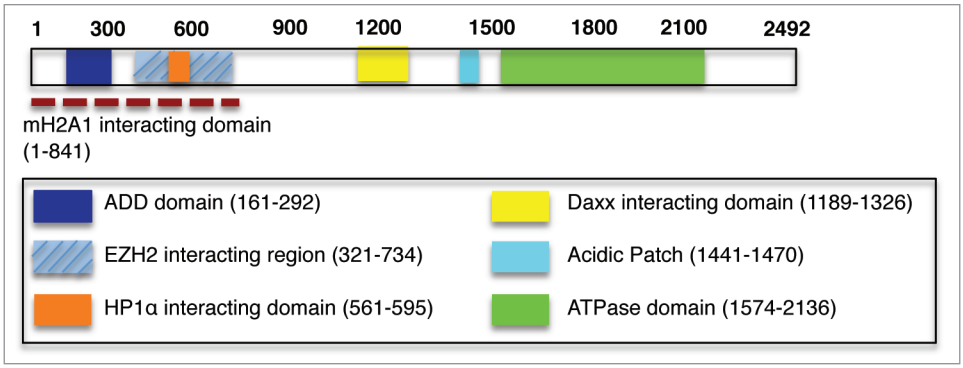

Figure 1. ATRX is a multidomain-containing chromatin remodeler. The ADD domain of ATRX (dark blue box) contains both a GATA-like domain and a PHD and "reads" the H3K9me3 modification.[6,21-23] We reported the in vivo interaction between the N-terminal 841 amino acids of ATRX with macroH2A1 (dashed red line).[19] ATRX interacts with HP1α via a PxVxL motif present in the indicated region (orange box).[16] Of note, while the interaction between EZH2 and ATRX (hatched blue box) was detected by yeast two hybrid,[17] it remains to be seen if this persists in vivo and if indeed the entire region depicted is required for this interaction.

ATRX and Histone H3 Interactions

ATRX has been linked to histone variant regulation by Choo and colleagues, who described the co-localization of ATRX and H3.3 at telomeres in mouse embryonic stem cells (ESCs).[14,24] This work also reported the loss of ATRX from telomeres upon differentiation concomitant with an increase of heterochromatic histone modifications, suggesting that ATRX facilitates a euchromatic state at telomeres.[14,24] Their work also showed that lysine 4 (K4) of H3.3 is critical for the in vivo interaction between ATRX and H3.3.[14] Subsequently, three reports emerged describing the direct binding of the ADD domain of ATRX to H3K9me3 via biochemical and structural studies.[21-23] This binding was perturbed by the transcriptionally active H3K4me3 modification, suggesting preferential association of ATRX with repressive chromatin. These studies further suggested that ATRX localization to heterochromatin is contingent upon H3K9me3, and we too have observed the in vivo association of ATRX with H3K9me3 (K.R. and E.B., unpublished data). Hence, the K4 residue reported to mediate the ATRX in vivo interaction by Choo and colleagues is likely due to K4 being a critical residue required for the ADD-H3 tail interaction.[14,21]

Goldberg et al. demonstrated a functional interaction between H3.3 and ATRX in mouse ESCs.[25] While HIRA had been characterized as a histone chaperone for H3.3,[26] Goldberg et al. observed that ATRX is also involved in H3.3 deposition. Interestingly, while HIRA deposits H3.3 at transcription start sites (TSSs) and within gene bodies, ATRX deposits H3.3 at telomeres. This study uncovered that distinct factors are required for site-specific deposition of H3.3, thereby highlighting the intricacy of histone variant deposition. We anticipate much more complexity in this system not only for H3.3, but also other histone variants.

ATRX has been reported to complex with the death-domain associated protein Daxx.[25,27,28] Drane et al. reported an ATRX and Daxx-dependent deposition of H3.3; however, this time at pericentric heterochromatin.[29] Both ATRX and Daxx were found enriched at major satellite repeats and it was suggested that H3.3 deposition at these regions was unlikely to facilitate heterochromatin formation but rather to drive transcription of pericentric repeats, thus implicating ATRX in transcriptional activation. We highlight here that recent evidence points toward Daxx as the direct histone chaperone for H3.3.[29,30] In fact, two structural studies resolved Daxx in complex with an H3.3-containing nucleosome and demonstrated that glycine 90, which is unique to H3.3 (as compared with H3.1 and H3.2), is crucial for this interaction.[31,32]

While future studies will allow a better understanding of the role of ATRX in heterochromatin regulation, a potential model begins to emerge from these studies. In the case of telomeres, ATRX assists Daxx in H3.3 deposition potentially to facilitate chromatin accessibility of highly replicating telomeres in ESCs.[29,30,33] As cells differentiate, ATRX is lost from telomeres allowing heterochromatin formation to ensue.[14,24] Consistent with these findings, the ATRX/Daxx dependent deposition of H3.3 at pericentric regions, mentioned above, is likely to facilitate an open environment conducive to the active transcription of pericentric repeats.[29] However, Goldberg et al. reported an increase in the presence of telomere repeat containing RNA (TERRA) in the absence of ATRX, suggesting that ATRX is also involved in transcriptional repression at telomeres.[25] Taken together, numerous questions arise from these studies: How does recognition of H3K9me3 via ATRX ADD domain contribute to such transcriptional activities? Does ATRX bind H3K9me3 on histone H3.3 specifically or other H3 family members as well? Does ATRX bind to heterochromatin to facilitate silencing, to potentially remodel chromatin for transcriptional activation, or to maintain distinctive but ill-defined chromatin states?

Histone Deposition at the α-*Globin* Locus

Our interest in the regulation of macroH2A chromatin association led to the identification of ATRX association with this transcriptionally repressive H2A variant.[19] In ATRX knockdown cells, which mimic the levels of ATRX protein found in ATRX syndrome patients,

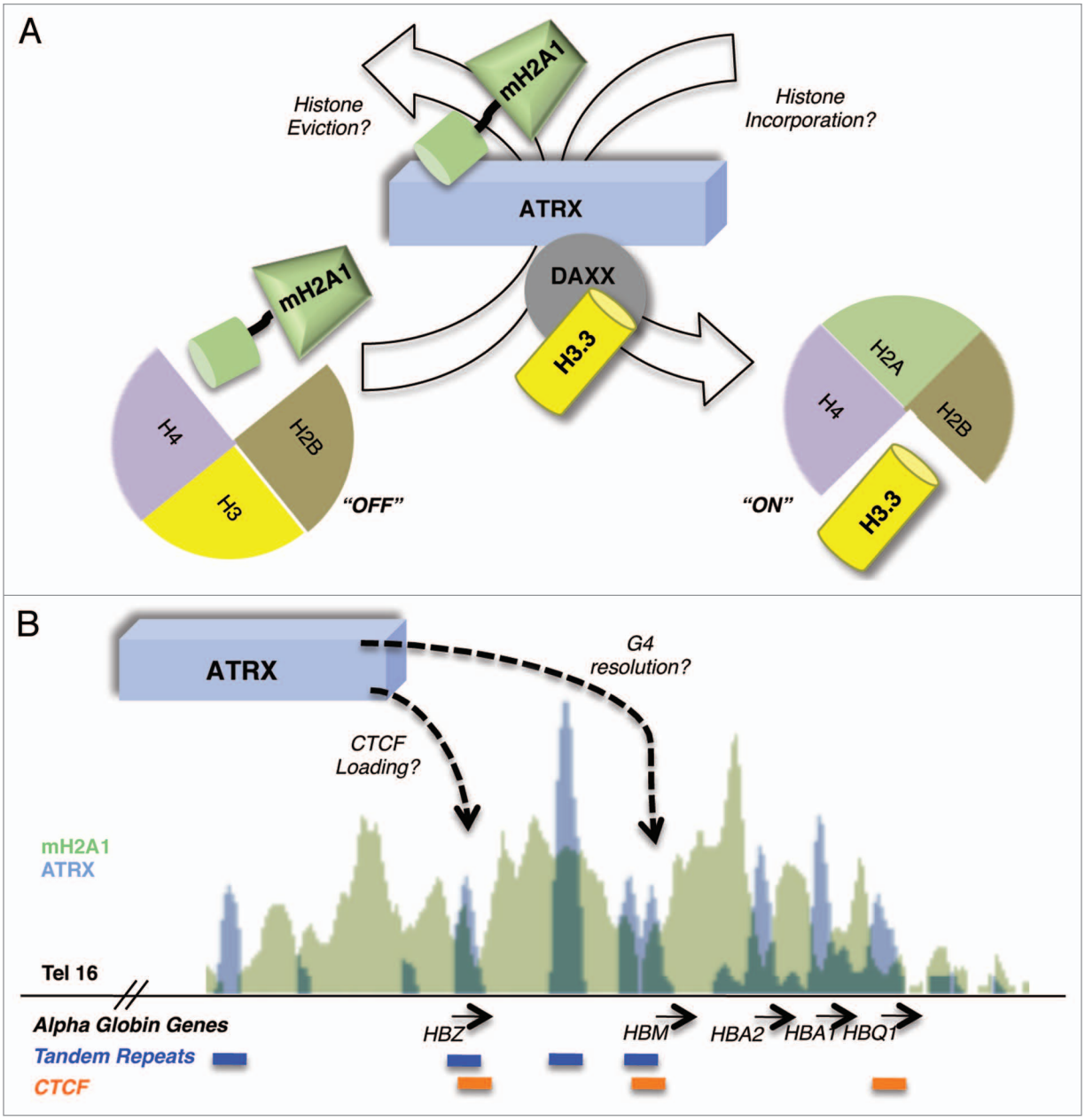

Figure 2. Hypothetical models for ATRX-mediated histone exchange and transcriptional activation of the α-*globin* gene cluster. (**A**) ATRX facilitates activation via the nucleosomal eviction of macroH2A1 and/or the Daxx mediated incorporation of H3.3 into nucleosomes potentially switching between "on" and "off" chromatin states. (**B**) ATRX loads CTCF and resolves repressive G4 DNA structures at tandem repeats (TRs) to permit gene activation at the α-*globin* cluster. Shown is a 50kb UCSC genome browser snapshot of the α-*globin* cluster. ChIP-sequencing data plots of macroH2A1 (K562 cells, green)[19] and ATRX (primary erythroblasts, blue)[12] are overlaid (scales are 70 and 600 respectively). Below the panel, genes are presented in black, ATRX associated tandem repeat tracks in blue and CTCF associated sites from genome wide ChIA-PET analysis (K562 cells)[43] in orange. All three CTCF sites overlap ATRX peaks and two of these occur at TRs. Of note, the ChIP-sequencing data presented were obtained from cell lines with different expression levels of α-globin (primary erythroblasts express α-globin at much higher levels than K562[57]) and the association of macroH2A1 and ATRX with the locus are likely reflective of the differences in α-*globin* transcription between these two cell types.

we observed increased levels of macroH2A1 globally and, more specifically, at telomeres and the α-*globin* gene cluster (proximal to human telomere 16). This corresponded to decreased α-globin expression. Furthermore, we reported an exclusive global localization pattern between ATRX and macroH2A1, suggestive of an antagonistic relationship (see **Fig. 2**). While ATRX is enriched at TSSs of active genes, macroH2A1 is excluded from the TSS and enriched upstream and/or in the gene bodies of inactive genes.[12,19]

Our studies suggest that ATRX negatively regulates the chromatin association of macroH2A, and support the notion of ATRX as transcriptional activator. Of note, while ATRX regulates H3.3 deposition via Daxx,[29,30] ATRX interacts with macroH2A in a Daxx-independent

manner.[19] We also reported a loss of expression of other genes outside of the α-*globin* cluster, suggesting that ATRX positively regulates genes in this greater genomic region in part by inhibition of deposition or through eviction/replacement of macroH2A (**Fig. 2**). It is now of interest to determine the mechanism by which ATRX regulates macroH2A deposition, and the H3.3 deposition changes that may occur at the α-*globin* cluster in the absence of ATRX. While HIRA was suggested to be the primary histone chaperone for H3.3 at genic regions in mouse ESCs,[25] the α-*globin* cluster is occupied by ATRX when actively transcribed, such as in primary erythroblasts,[12] suggesting that ATRX might mediate H3.3 deposition here.

We also observed that the N-terminal region of ATRX (1–841), containing the ADD domain, was sufficient for interaction with macroH2A (**Fig. 1**).[19] This raises the enticing model that ATRX recognizes nucleosomes containing H3K9me3 (on H3.1 and H3.2) via its ADD domain (**Fig. 1**) and assists in the exchange of macroH2A for H3.3 (via Daxx) into nucleosomes at the α-*globin* locus, and other genomic regions such as telomeres (**Fig. 2**). This would promote a switch toward open chromatin, and may help to reconcile ATRX heterochromatin localization with its conflicting role in gene activation.

α-Thalassemia Syndrome Variability—G Quadruplex Structures

While the regulation of histone variant deposition by ATRX supports a direct role in transcriptional activation, recent work from Higgs and colleagues suggested a novel mechanism by which ATRX supports activation.[12] In a study aimed at understanding the variability of phenotypes among patients bearing the same *ATRX* gene mutations, ChIP-sequencing studies revealed that ATRX primarily occupies G-rich tandem repeats (TRs) which can form G quadruplex (G4) DNA structures in vivo. It was suggested that the differences in size of these TRs among patients contributes to the ranges in severity of the syndrome. For example, TRs were reported to be at or around the α-*globin* cluster and the absence of ATRX resulted in a distance-dependent silencing of genes (including the α-*globin* genes). Those genes closest to the TRs were the most severely affected. Higgs and colleagues' model proposes that ATRX facilitates transcription, indirectly, by binding to and resolving potentially repressive G4 structures. They further hypothesized that this involves the incorporation of the variant H3.3. It would indeed be interesting to test if the incorporation of the variant H3.3 and/or the removal of macroH2A are involved in the putative helicase-mediated resolution of G4 structures (**Fig. 2**). While this study points toward a role for ATRX in transcriptional activation, recent reports described below may complicate this model or suggest that each genomic region has to be assessed individually for ATRX function.

Transcriptional Repressor, Transcriptional Activator or Something in Between?

The ability to visualize ATRX and Daxx proteins at an inducible multi-copy transgene array in single cells has allowed analysis of their role in regulating chromatin states.[34,35] In the presence of ATRX and Daxx, the array is refractory to transcriptional activation and upon induced activation both proteins are lost, implicating them in the maintenance of a repressive chromatin state. The array displayed robust activation in the ATRX-negative U2OS osteosarcoma cell line and addition of exogenous ATRX resulted in reduced transcriptional activation. This was ascribed, at least in part, to its helicase activity, suggesting that while the more N-terminal portion of ATRX recognizes histone modifications and associates either directly or indirectly with histone variants, its C-terminal helicase domain has broader roles in regulating chromatin structure. This remodeling activity may be dependent on ATRX locus-specific protein interactions or association with histone variants.

A study performed in *Drosophila melanogaster* examined the ATRX homolog, XNP. Ahmad and colleagues analyzed the gain- and loss-of-function phenotypes of XNP using the fly eye, and found that both produced the same effect—de-repression of silencing.[36] Taken together, it might appear that ATRX maintains a balance between the two chromatin states (heterochromatic and euchromatic) and changes in its levels, in either direction, result in an alteration of chromatin structure, possibly in a locus-specific manner. In this case, a loss of heterochromatin was observed.[36] While very intriguing, a caveat here is that Drosophila XNP does not possess an ADD domain,[37] which might confound these studies when applied directly to mammalian systems.

ATRX—a Regulator of Chromatin Domains?

Kernohan et al. suggested that ATRX is involved in the loading of the insulator protein, CTCF, onto the H19 gene locus in the mouse brain—in ATRX knock out cells, a decreased association of the CTCF protein was observed.[18] The CTCF protein has long been described to act as a barrier between chromatin domains[38-40] and hence its absence could result in aberrant spreading and/or deposition of histones with opposing transcriptional activity.[41] Gamble et al. analyzed macroH2A1 genome-wide deposition pattern and determined that CTCF is enriched at macroH2A1 domain boundaries.[42] Furthermore, the ENCODE genome project reveals several sites of overlap between ATRX and CTCF at the α-*globin* cluster (**Fig. 2**).[12,43] We question: is the loss of H3.3 or the increased deposition of macroH2A1 a product of a defective chromatin barrier created in part by aberrant CTCF deposition at particular genomic loci in an ATRX-deficient background (**Fig. 2**)? Therefore, it will be of interest to study alterations to histone deposition patterns in ATRX patients. While the α-thalassemia phenotype is well characterized to occur via transcriptional alterations at the α-*globin* cluster, the mental retardation aspects of ATRX are poorly understood. Studying the genomic alterations of histones and factors that influence the chromatin template in ATRX patients will likely shed light on additional mechanisms involved in this syndrome.

Concluding Remarks

The numerous observations pertaining to ATRX suggest that we have only begun to uncover its roles in transcriptional regulation, and we look forward to studies that resolve the questions we raise herein. Moreover, we anticipate exciting new studies that examine ATRX in the context of tumor biology. Curiously, *ATRX* mutations and deletions have been reported in varied tumor types including pancreatic neuroendocrine tumors (panNETs), pediatric neuroblastoma and glioblastoma, as well as the rare α-thalassemia myelodysplasia syndrome (ATMDS).[44-51] While the mechanism is yet unknown, it is likely that *ATRX* mutations in the context of tumor biology are distinct to those in ATRX syndrome, as patients with ATRX syndrome have not been reported to be pre-disposed to the afore mentioned tumors.[44-52]

Given the discussion here, we hypothesize that *ATRX* mutations are directly involved in tumorigenesis via alterations at the chromatin level and genome-wide analyses of histone variant deposition and histone post-translational modifications (PTMs) in the context of these tumors will elucidate whether this is indeed the case. ATRX has also been implicated in sister chromatid cohesion and mutations to ATRX potentially contribute to tumorigenesis via aberrant chromosome dynamics.[53] As there are apparent roles for ATRX in telomere homeostasis,[14,19,25,54] it is now of interest to determine the perturbations to histones, particularly H3.3 and macroH2A, in the absence of functional ATRX and if/how this might contribute to tumorigenesis via telomere dysfunction. Additionally, ATRX has been shown to localize to promyelocytic leukemia (PML) nuclear bodies, together with Daxx,[27] and while it is unclear if there is a connection between PML bodies and histone regulation, a full understanding of the varied cellular roles of ATRX will certainly involve delineating its connection to PML bodies.

ATRX has been directly linked to repression in some cases, while in others it appears to be intimately associated with gene activation. Work from Berube and colleagues reported phosphorylation-dependent changes to ATRX localization during the cell cycle.[55] In particular, ATRX was present at the nuclear matrix in interphase, but observed along chromosomes during mitosis when it interacts with HP1α. The authors suggested dual roles for ATRX in gene expression (interphase) and chromosome segregation (mitosis), as distinguished by its PTM profiles. Perhaps attempts at delineating ATRX function in transcription will lead to the identification of differentially modified forms of ATRX that have specific binding partners, thereby conferring distinct functions.

While an intriguing extrapolation that requires testing, the possibility that ATRX regulates a more global genome association of CTCF (than just the HG19 locus[18]) is particularly enticing. ATRX might then be involved in a greater organization of the genome than has been previously thought potentially via regulation of chromatin domains and higher order chromatin structure.[56]

Finally, we imagine that genome-wide localization studies of ATRX, as well as biochemical analyses in different cell types (e.g., erythroid, neuronal, tumor cells) will add to our growing knowledge about this peculiar helicase and how its function goes awry in disease. Does ATRX localize to the same genomic regions in erythroid cells as it does in neuronal cell types? Does ATRX interact with Daxx (and thereby H3.3) and macroH2A in all cell types or does it exhibit tissue specificity? In *ATRX* mutated tumors that retain nuclear ATRX expression or express truncated forms of the protein, what does the epigenomic landscape look like? How are histone variants and histone PTMs altered, if at all, in the absence of functional ATRX? These questions are indeed of critical and timely importance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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