Review

The chromatin remodeller ATRX: a repeat offender in human disease

David Clynes, Douglas R. Higgs, and Richard J. Gibbons

MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, UK

The regulation of chromatin structure is of paramount importance for a variety of fundamental nuclear processes, including gene expression, DNA repair, replication, and recombination. The ATP-dependent chromatin-remodelling factor ATRX (α thalassaemia/mental retardation X-linked) has emerged as a key player in each of these processes. Exciting recent developments suggest that ATRX plays a variety of key roles at tandem repeat sequences within the genome, including the deposition of a histone variant, prevention of replication fork stalling, and the suppression of a homologous recombination-based pathway of telomere maintenance. Here, we provide a mechanistic overview of the role of ATRX in each of these processes, and propose how they may be connected to give rise to seemingly disparate human diseases.

ATRX: from thalassaemia to cancer

To understand the molecular mechanisms underlying many human diseases we need to consider DNA in the context of chromatin. Chromatin consists of 147 bp of DNA wrapped around a histone octamer that is subsequently assembled into compacted higher-order structures. The role of chromatin extends far beyond that of a simple packaging tool; it is post-translationally modified, facilitating the recruitment of binding partners, and its structure is dynamically regulated, allowing for the modulation of a wide variety of biological processes (for extensive reviews see [1,2]). The identification of human diseases caused by mutations in genes that encode proteins required for the remodelling or epigenetic modification of chromatin has underscored the importance of chromatin structure in human disease. One such disorder, ATR-X syndrome, is caused by mutations in the *ATRX* gene. *ATRX* encodes a chromatin remodeller (ATRX), which is a 280-kDa protein that includes an unusual N-terminal plant homeodomain (PHD) designated the ATRX-DNMT3-DNMT3L (ADD) domain owing to its similarity to a protein region found in the DNA methyl transferases (DNMTs) [3–5]. Located at the C terminus are seven helicase subdomains that confer ATPase activity and identify ATRX as a snf2 family member of chromatin-associated proteins; many of which characteristically slide, remodel, or remove histones in *in vitro* assays [6]. It is now known that ATRX, in collaboration with its interaction partner death-associated protein 6 (DAXX), functions as a histone chaperone complex for the deposition of the histone variant H3.3 into pericentric, telomeric, and ribosomal repeat sequences [7–10].

ATR-X syndrome is characterised by a variety of clinical features that include mental retardation, facial, skeletal, and urogenital abnormalities, as well as mild α-thalassaemia (a blood disorder characterised by an imbalance of globin chain synthesis and anaemia) [11,12]. The latter is attributable to reduced expression of the α globin genes located on chromosome 16. ATRX was hence considered to be an X-chromosome-encoded *trans*-acting factor that facilitates the expression of a select repertoire of disparate genes. Subsequent studies in several model organisms have since uncovered defects in multiple important cellular processes upon perturbation of ATRX function, including defective sister chromatid cohesion and congression [13,14], telomere dysfunction [15], and aberrant patterns of DNA methylation (at rDNA, subtelomeric, and heterochromatic repeats [16]). Exciting recent developments have also implicated loss of ATRX function in a specific subset of malignancies that depend on a telomerase-independent pathway of telomere maintenance called the 'alternative lengthening of telomeres' (ALT) pathway [17–19]. Such a broad spectrum of pathologies led us to speculate as to whether the underlying molecular mechanisms behind these pathologies are interrelated. Here, we explore this possibility by outlining the recent advances in our understanding of ATRX cellular function and what this implies about the role of chromatin in human disease.

ATRX: a repeat offender on heterochromatin

A prominent clue to the physiological role of ATRX has come from studying the distribution of ATRX in the nucleus. Indirect immunofluorescence studies have revealed that ATRX has a strong preference for binding within promyelocytic leukaemia (PML) bodies and also to repetitive heterochromatic regions such as rDNA, telomeric, and pericentric DNA repeats [16,20,21].

Previous work indicates that localisation of ATRX to heterochromatin involves an interaction with the heterochromatin protein HP1 and in neuronal cells methyl cpg binding protein 2 (MeCP2) is also implicated [22,23]. Furthermore, targeting of ATRX to pericentric heterochromatin is dependent on trimethylation of histone H3 at Lys9. Knockout of the methyltransferases Suv39H1 and Suv39H2, which are responsible for 'writing' this

Corresponding author: Gibbons, R.J. (richard.gibbons@imm.ox.ac.uk).
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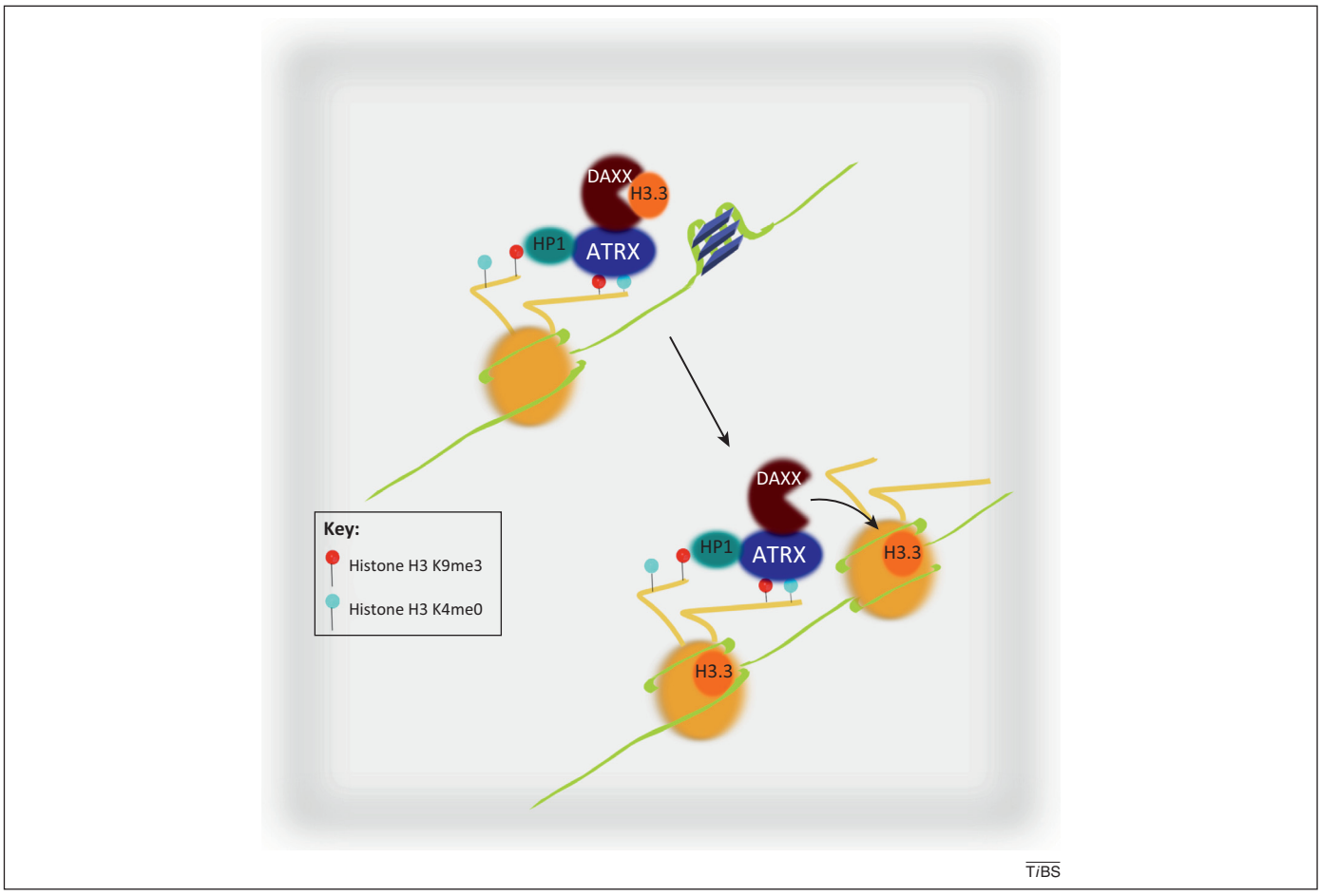

Figure 1. Recruitment of ATRX (α-thalassaemia/mental retardation X-linked) to chromatin. Binding of ATRX to histone H3 at heterochromatic sites occurs through interaction of the ATRX ADD (ATRX-DNMT3-DNMT3L) domain with a histone H3 N-terminal tail that is trimethylated at Lys9 and unmodified at Lys4. Recruitment is enhanced by a third interaction through heterochromatin protein (HP)1 that also recognises trimethylated H3 Lys9 [24,27] (left). Once recruited to its target sites ATRX, in combination with its interaction partner death-associated protein 6 (DAXX), facilitates the deposition of the histone variant H3.3 [7–10], which may maintain DNA in the B-form [57].

modification, completely abrogates its recruitment [24]. An appealing hypothesis to emerge from these two observations is that HP1 might recruit ATRX to pericentric heterochromatin indirectly by serving as a protein scaffold, facilitating the recruitment of ATRX through binding of H3 K9me3 via its N-terminal chromodomains [25]. A major caveat to this hypothesis, however, is that it provides limited specificity given that HP1 is a widely distributed, constitutive component of heterochromatin with a large repertoire of binding partners [26]. Recent work by Eustermann *et al*. and Iwase *et al*. elegantly addresses this question by showing that in addition to binding HP1, ATRX binds directly to the histone H3 tail. Importantly, specificity for this interaction is governed through two distinct binding pockets located in the N-terminal ADD domain; one accommodating unmodified Lys4 and the other restricted to di- or trimethylated Lys9. This allows for a combinatorial readout of K4me0 and K9me3 (or K9me2) on the histone H3 N-terminal tail, with recruitment to K9me3 further enhanced by the previously characterised interaction with HP1 [24,27] (Figure 1). ATRX recruitment to heterochromatin thereby adds credence to the existence of a multi-faceted 'histone code' that is 'read' by a combination of multivalent effector–chromatin interactions. The importance of the ATRX ADD domain in the underlying aetiology of ATR-X syndrome is highlighted by the finding that it serves as a 'hot spot' for syndrome-associated mutations [28].

ATRX: a histone chaperone

Once recruited to its target sites, how does ATRX ensure appropriate gene expression and/or maintain genomic stability? It is now known that ATRX limits the deposition of the histone variant macroH2A1 [29] (Box 1), whereas, in combination with its interaction partner (DAXX), it can facilitate the incorporation of another histone variant, H3.3, into pericentric, telomeric, and ribosomal repeat sequences [7–10]. DAXX functions as a highly specific histone chaperone that is able to discriminate H3.3 from

Box 1. ATRX: sequestration of a histone variant

Recent research has identified an interaction between ATRX and another histone variant, macroH2A1 [29]. MacroH2A1 is generally considered to associate with transcriptionally inert chromatin and is frequently found associated with heterochromatin [60]. Ratnamukar *et al.* have shown that, in contrast to the role of ATRX/DAXX in histone H3.3 deposition, ATRX acts as a negative regulator of macroH2A chromatin association. Knockdown of ATRX results in an increase in the accumulation of macroH2A1 at telomeres and at the α-globin cluster in a human erythroleukaemia cell line, suggesting that ATRX may additionally promote α-globin expression by maintaining chromatin in an active configuration through sequestration of macroH2A1.