

1 **The chromatin remodeller ATRX: A repeat offender in human disease**

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Abstract

The regulation of chromatin structure is of paramount importance for a variety of fundamental nuclear processes including; transcription, DNA repair, replication and recombination. The ATP-dependent chromatin-remodeling factor 'ATRX' (alpha thalassemia/mental retardation X-linked) has emerged as a key player in each of these processes. Exciting recent developments suggest that ATRX plays a repertoire of key roles at tandem repeat sequences within the genome; including the deposition of a histone variant, DNA replication, 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, discuss how they may be connected and their relationship to human disease.

ATRX: From thalassemia 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 which is then subsequently assembled into compacted higher order structures. The role of chromatin extends far beyond that of a simple packaging tool, its structure is dynamically regulated allowing for the modulation of a wide variety of biological processes. The importance of chromatin structure in human disease has become increasingly apparent through the identification of human diseases caused by mutations in genes that encode proteins required for the remodeling of, or epigenetic modification of chromatin. One such disorder 'ATR-X syndrome' is caused by mutations in the *ATRX* gene, of which approximately 127 unique mutations have been identified. *ATRX* encodes a

chromatin remodeler (ATRX) which is a 280 kDa protein that includes an unusual N-terminal plant homeodomain (PHD) designated the ATRX-DNMT3-DNMT3L (ADD) [1] domain owing to its similarity to a protein region found in this group of methyl transferases [2-4]. Located at the C-terminus are seven helicase subdomains, conferring ATPase activity and identifying ATRX as a snf2 family member of chromatin associated proteins, many of which characteristically slide, remodel or remove histones in *in vitro* assays [5]. It is now known that ATRX, in collaboration with its interaction partner DAXX, functions as a histone chaperone complex for the deposition of the histone variant H3.3 [6-9].

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 -thalassemia [10, 11]. The latter is attributable due to reduced expression of the alpha globin genes located on chromosome 16. ATRX was hence considered to be an X encoded *trans*-acting factor that facilitates the transcription of a diverse 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 [12, 13], telomere dysfunction [14] and aberrant patterns of DNA methylation (at ribosomal DNA, subtelomeric and heterochromatic repeats [15]). Exciting recent developments have also implicated loss of ATRX function in a specific subset of malignancies that depend on a telomerase independent-based pathway of telomere maintenance, 'the Alternative Lengthening of Telomeres (ALT)' pathway [16-18]. Such a broad spectrum of pathologies raises interesting questions as to whether the underlying molecular mechanisms behind these pathologies are interrelated. Here

we address this question by outlining the recent advances made in our understanding of ATRX cellular function and what this tells us 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 to repetitive heterochromatic regions; at rDNA repeats, telomeric repeats, pericentric DNA repeats and within promyelocytic leukaemia (PML) bodies [15, 19, 20]. The advent of deep sequencing technologies expanded on these observations by showing that ATRX has an overwhelming preference to bind tandem repetitive (TR) DNA, especially G-rich repeats as well as CpG islands (regions of the genome with a high frequency of unmethylated cytosine guanine dinucleotides) [21].

Further work has subsequently given mechanistic insight as to how ATRX is recruited to heterochromatin. Yeast two hybrid screens have shown that ATRX interacts with a murine homologue (mHP1 α) of the *Drosophila* Heterochromatic protein HP1 [22]. Furthermore, targeting of ATRX to pericentric heterochromatin was found to be dependent on trimethylation of histone H3 at Lys9 on its N-terminal tail. Knockout of the methyltransferases Suv39H1 and Suv39H2, responsible for 'writing' this modification completely abrogate its recruitment [23]. An appealing hypothesis to emerge from these two observations was 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 [24]. A

major caveat to this hypothesis, however, is that it would provide limited specificity given that HP1 is a widely distributed, constitutive component of heterochromatin with a large repertoire of binding partners [25]. 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 [26, 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 etiology of ATR-X syndrome is highlighted by the finding that it serves as a 'hot spot' for syndrome-associated mutations [28].

Recruitment of ATRX to its target sites may not, however, be restricted to its interaction with the histone H3 tail. The favoured target of ATRX, G-rich tandem repetitive DNA, has the potential to adopt a range of non-canonical DNA conformations. Of particular note, approximately 50% of ATRX target sites, including telomeres, are predicted to adopt the G-quadruplex conformation [21] and there is mounting evidence that these structures form *in vivo* [29-31]. Electrophoretic mobility shift assays have demonstrated that recombinant ATRX protein preferentially binds to G4-structured DNA over an unfolded telomere-derived sequence [21], strongly suggesting ATRX may directly bind these DNA structures *in vivo*.

101 **ATRX: a histone chaperone**

102 Once recruited to its target sites how does ATRX ensure appropriate gene
103 expression and/or maintain genomic stability? It is now known that ATRX, in
104 combination with its interaction partner DAXX, facilitates incorporation of the histone
105 variant H3.3 into pericentric, telomeric and ribosomal repeat sequences [6, 8, 9, 32].
106 DAXX functions as a highly specific histone chaperone, able to discriminate H3.3
107 from the other major histone H3 variants H3.1 and H3.2, whereas ATRX is involved
108 in targeting DAXX to repetitive sequences and enhancing this deposition [6, 8, 32].
109 Understanding the function of H3.3 at ATRX target sequences is therefore likely to
110 prove pivotal in understanding ATRX function in relation to human disease. In
111 contrast to the canonical histones, H3.3 is synthesised throughout the cell cycle and
112 predominantly undergoes replication-independent deposition [33, 34]. H3.3 was
113 traditionally considered as a marker for highly dynamic, active chromatin and its
114 deposition in heterochromatic regions might therefore seem somewhat counter-
115 intuitive. Repetitive DNA, however, is predicted to be an inherently “unfriendly”
116 substrate for nucleosomes, leading to rapid turnover. ATRX/DAXX-dependent H3.3
117 deposition may be necessary to stabilise a B-DNA conformation in tandem repetitive
118 ATRX target sites. This raises the question of whether the pathologies observed
119 upon loss of ATRX function could be at least partially attributable to an increase in
120 structured DNA at these sites?

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124 **ATRX and alpha thalassemia**

125 ATR-X syndrome is characteristically associated with alpha-thalassemia, attributable
126 to the down-regulation in expression of the alpha-globin genes located close to the
127 telomere of chromosome 16. ChIP-seq data suggest that tandem repetitive DNA
128 does indeed have a crucial role to play in this phenomenon by showing that instead
129 of binding directly to the alpha globin genes, ATRX localises several kb upstream
130 within a TR sequence, denoted $\Psi\zeta$. Strikingly, in ATR-X syndrome patients it was
131 observed that the genes immediately proximal to this repeat were the most down
132 regulated, and those furthest away (~10Kb) the least perturbed. It was further noted
133 that the size of the $\Psi\zeta$ TR varies between patients and the degree of thalassemia
134 was proportional to the length of the $\Psi\zeta$ TR, explaining the variable degrees of
135 thalassemia observed between patients with identical ATRX mutations [21]. The $\Psi\zeta$
136 TR adopts a G-quadruplex conformation *in vitro* and a plausible explanation for this
137 observation is that the longer the TR sequence, the higher the probability that it will
138 adopt a non-canonical secondary structure such as a G-quadruplex. Consistent with
139 such a hypothesis previous studies have shown that the length of a G-quadruplex
140 forming minisatellite, CEB1, is related to its instability upon deletion of the known G-
141 quadruplex resolving helicase, Pif1, in yeast cells [35].

142 **ATRX and DNA replication**

143 Assuming such a hypothesis is correct, it raises the intriguing question as to how the
144 presence of an aberrant DNA secondary structure may lead to the transcriptional
145 silencing of genes located several Kb downstream of the TR DNA. Structured DNA,
146 such as G-quadruplexes, are known to form barriers to several nuclear processes,

147 including DNA replication and transcription [31, 36, 37]. Consistent with this, recent
148 studies have shown that loss of ATRX function is associated with replication defects,
149 as identified by an increase in replication fork stalling, a prolongation in S-phase and
150 concomitant accumulation of p53 and the γ -H2AX DNA damage marker, particularly
151 at telomeres, a known site for G-quadruplex formation and ATRX binding [14, 38, 39].
152 Accumulating evidence is emerging that problems in replicating structured DNA can
153 also cause perturbations in local gene expression. The accurate propagation of
154 histone marks during chromosomal replication is thought to rely on the tight coupling
155 of replication with the recycling of parental histones. Work by Sarkies et al., has
156 shown that interruption of processive DNA replication at G4 sequences in cells
157 lacking various G4 helicases results in this tight coupling being lost, leading to the
158 biased incorporation of newly synthesised histones (associated with H4 N-terminal
159 acetylation but not other marks of activation or repression). Genes that were
160 previously active became repressed and conversely genes that were previously
161 repressed became active [40, 41]. One could also envisage an alternative
162 explanation attributable to epigenetic silencing arising from the creation of a double
163 strand break, which is known to be generated upon collapse of a stalled replication
164 fork (for comprehensive reviews see [42, 43]). Work by the Greenberg laboratory has
165 recently demonstrated such a phenomenon by showing that the induction of a
166 double strand break can lead to the silencing of a reporter gene some 4kb
167 downstream from the break site through H2A ubiquitylation dependent pausing of
168 RNAPII [44] (Figure 2). Further work is needed to dissect whether either of these
169 events are indeed occurring at the alpha-globin locus in ATR-X syndrome, through

careful profiling of DNA replication through the associated $\Psi\zeta$ TR and the detection of any associated epigenetic changes arising from this site.

Given that ATRX appears to have general roles in the deposition of a histone variant and DNA replication, one might expect that the cellular consequences of losing ATRX function would include a loss of genome stability and chromosomal congression defects. In the appropriate cellular context this does indeed appear to be the case, with loss of ATRX associated with extensive genomic rearrangements, DNA damage and telomere fusions [14, 18, 38]. Furthermore, loss of ATRX function has been shown to result in both mitotic and meiotic defects, including defective spindle formation, chromosome condensation and alignment [12, 13, 38]. A link between defective replication and chromosome condensation has long been established (for review see [45]) and it is therefore possible that these phenotypes may, at least partially be attributable to the DNA replication defects observed upon loss of ATRX function.

ATRX and Cancer

Of particular recent interest is the finding that mutations in ATRX are associated with a specific subgroup of tumours that employ a telomerase-independent mechanism of telomere maintenance, termed the Alternative Lengthening of Telomeres (ALT) pathway. To grow indefinitely, cancer cells must circumvent the progressive attrition of telomeric DNA that inevitably occurs with each cell division, ultimately leading to cellular senescence or crisis. In the majority of cancer cells this is potentiated through re-activation of telomerase, a specialised reverse transcriptase that synthesises the tandem TTAGGG hexanucleotide repeat by using its own RNA

subunit as a template [46, 47]. However, 10 – 15% of cancers lack detectable telomerase and are instead reliant on a telomere maintenance pathway that depends on homologous recombination (HR) between telomeric sequences (for extensive reviews see [48, 49]). Strikingly, a recent study has documented that loss of ATRX is a trait of 90% of *in vitro* immortalised ALT cell lines [18]. Moreover, mutations in ATRX and/or DAXX have been identified in many tumours exhibiting ALT, including pancreatic neuroendocrine tumours, pediatric glioblastomas, oligodendriogomas and medulloblastomas [16]. More recently additional mutations in H3.3 have been identified in ALT-positive paediatric glioblastomas [17], strongly suggesting that the ATRX/DAXX/H3.3 pathway has a role in normally suppressing the ALT pathway.

Increased stalling of replication forks, a known trigger of HR, at telomeric sites may contribute to the initiation of aberrant telomeric recombination and activation of the ALT pathway in the absence of functional ATRX. Telomeres are known to be inherently difficult sites to replicate and resemble fragile sites [50] and the stabilisation of G-quadruplex structures using a stabilising ligand has been shown to perturb DNA replication at telomeres and trigger a DNA damage response [37]. This implies that an increased presence of DNA secondary structure at telomeres might lead to increased replication fork stalling. Furthermore, the unidirectional nature of telomere replication [50] means that a converging fork cannot salvage a stalled replication fork, presumably increasing the reliance on HR mediated fork restart (Figure 2).

Several unanswered questions remain. Knockdown of ATRX in a telomerase positive cell line is not sufficient to trigger the ALT pathway [18]. The cellular context,

216 including the telomerase-negative status of ALT cells, must therefore be an
217 important additional factor in the development of the ALT pathway and more
218 research will be required to determine what additional factors are required to trigger
219 ALT in an ATRX null genetic landscape.

220 **Summary**

221 The study of ATR-X syndrome illustrates the benefit of studying rare genetic
222 disorders and highlights the importance of chromatin remodellers in human disease.
223 From gaining a detailed molecular insight as to how and where ATRX is recruited in
224 the genome and identifying the ATRX/DAXX complex as a novel histone chaperone
225 for H3.3 we have now begun to understand how ATRX regulates gene expression
226 and gained insight into how ATRX functions as an important tumour suppressor.
227 Surprisingly, insights gained from the study of one disorder can be applied to
228 understand the role of ATRX in seemingly disparate pathologies, highlighting the
229 importance of combining basic research with clinical genetics.

230 **Box 1.**

231 Recent research has also identified an interaction between ATRX and another
232 histone variant, macroH2A1 [51]. MacroH2A1 is generally considered to associate
233 with transcriptionally inert chromatin and is frequently found associated with
234 heterochromatin [52]. Ratnamukar et al. show that in contrast to the role of
235 ATRX/DAXX in histone H3.3 deposition, ATRX acts as a negative regulator of
236 macroH2A chromatin association. Knockdown of ATRX resulted in an increase in the
237 accumulation of macroH2A1 at telomeres and at the alpha globin cluster in a human
238 erythroleukemia cell line, inferring that ATRX may additionally promote alpha-globin

expression by maintaining chromatin in an active configuration through sequestration of macroH2A1.

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Reference list

1. Khachatryan, V., et al., *Search for Quark Compositeness with the Dijet Centrality Ratio in pp Collisions at root s=7 TeV*. Physical Review Letters, 2010. **105**(26).
2. Argentaro, A., et al., *Structural consequences of disease-causing mutations in the ATRX-DNMT3-DNMT3L (ADD) domain of the chromatin-associated protein ATRX*. Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(29): p. 11939-44.
3. Ooi, S.K., et al., *DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA*. Nature, 2007. **448**(7154): p. 714-7.
4. Otani, J., et al., *Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain*. EMBO Rep, 2009. **10**(11): p. 1235-41.
5. Picketts, D.J., et al., *ATRX encodes a novel member of the SNF2 family of proteins: mutations point to a common mechanism underlying the ATR-X syndrome*. Hum Mol Genet, 1996. **5**(12): p. 1899-907.

- 260 6. Lewis, P.W., et al., *Daxx is an H3.3-specific histone chaperone and cooperates with*
261 *ATRX in replication-independent chromatin assembly at telomeres*. Proceedings of
262 the National Academy of Sciences of the United States of America, 2010.
263 **107**(32): p. 14075-80.
- 264 7. Elsaesser, S.J., A.D. Goldberg, and C.D. Allis, *New functions for an old variant: no*
265 *substitute for histone H3.3*. Curr Opin Genet Dev, 2010. **20**(2): p. 110-7.
- 266 8. Drane, P., et al., *The death-associated protein DAXX is a novel histone chaperone*
267 *involved in the replication-independent deposition of H3.3*. Genes & development,
268 2010. **24**(12): p. 1253-65.
- 269 9. Goldberg, A.D., et al., *Distinct factors control histone variant H3.3 localization*
270 *at specific genomic regions*. Cell, 2010. **140**(5): p. 678-91.
- 271 10. Gibbons, R.J., et al., *Mutations in a putative global transcriptional regulator cause*
272 *X-linked mental retardation with alpha-thalassemia (ATR-X syndrome)*. Cell, 1995.
273 **80**(6): p. 837-45.
- 274 11. Gibbons, R.J., D.J. Picketts, and D.R. Higgs, *Syndromal mental retardation due to*
275 *mutations in a regulator of gene expression*. Hum Mol Genet, 1995. **4 Spec No**: p.
276 1705-9.
- 277 12. Ritchie, K., et al., *Loss of ATRX leads to chromosome cohesion and congression*
278 *defects*. The Journal of cell biology, 2008. **180**(2): p. 315-24.
- 279 13. De La Fuente, R., et al., *ATRX, a member of the SNF2 family of helicase/ATPases, is*
280 *required for chromosome alignment and meiotic spindle organization in*
281 *metaphase II stage mouse oocytes*. Developmental biology, 2004. **272**(1): p. 1-14.

- 282 14. Wong, L.H., et al., *ATRX interacts with H3.3 in maintaining telomere structural*
283 *integrity in pluripotent embryonic stem cells*. Genome research, 2010. **20**(3): p.
284 351-60.
- 285 15. Gibbons, R.J., et al., *Mutations in ATRX, encoding a SWI/SNF-like protein, cause*
286 *diverse changes in the pattern of DNA methylation*. Nature genetics, 2000. **24**(4):
287 p. 368-71.
- 288 16. Heaphy, C.M., et al., *Altered telomeres in tumors with ATRX and DAXX mutations*.
289 Science, 2011. **333**(6041): p. 425.
- 290 17. Schwartzentruber, J., et al., *Driver mutations in histone H3.3 and chromatin*
291 *remodelling genes in paediatric glioblastoma*. Nature, 2012. **482**(7384): p. 226-31.
- 292 18. Lovejoy, C.A., et al., *Loss of ATRX, Genome Instability, and an Altered DNA Damage*
293 *Response Are Hallmarks of the Alternative Lengthening of Telomeres Pathway*.
294 PLoS genetics, 2012. **8**(7): p. e1002772.
- 295 19. McDowell, T.L., et al., *Localization of a putative transcriptional regulator (ATRX)*
296 *at pericentromeric heterochromatin and the short arms of acrocentric*
297 *chromosomes*. Proceedings of the National Academy of Sciences of the United
298 States of America, 1999. **96**(24): p. 13983-8.
- 299 20. Xue, Y., et al., *The ATRX syndrome protein forms a chromatin-remodeling complex*
300 *with Daxx and localizes in promyelocytic leukemia nuclear bodies*. Proceedings of
301 the National Academy of Sciences of the United States of America, 2003.
302 **100**(19): p. 10635-40.

- 303 21. Law, M.J., et al., *ATR-X Syndrome Protein Targets Tandem Repeats and*
304 *Influences Allele-Specific Expression in a Size-Dependent Manner.* Cell, 2010.
305 **143**(3): p. 367-78.
- 306 22. Le Douarin, B., et al., *A possible involvement of TIF1 alpha and TIF1 beta in the*
307 *epigenetic control of transcription by nuclear receptors.* The EMBO journal, 1996.
308 **15**(23): p. 6701-15.
- 309 23. Eustermann, S., et al., *Combinatorial readout of histone H3 modifications specifies*
310 *localization of ATRX to heterochromatin.* Nat Struct Mol Biol, 2011. **18**(7): p. 777-
311 82.
- 312 24. Nielsen, P.R., et al., *Structure of the HP1 chromodomain bound to histone H3*
313 *methylated at lysine 9.* Nature, 2002. **416**(6876): p. 103-7.
- 314 25. Lechner, M.S., et al., *The mammalian heterochromatin protein 1 binds diverse*
315 *nuclear proteins through a common motif that targets the chromoshadow domain.*
316 Biochemical and biophysical research communications, 2005. **331**(4): p. 929-37.
- 317 26. Eustermann, S., et al., *Combinatorial readout of histone H3 modifications specifies*
318 *localization of ATRX to heterochromatin.* Nature structural & molecular biology,
319 2011. **18**(7): p. 777-82.
- 320 27. Iwase, S., et al., *ATRX ADD domain links an atypical histone methylation*
321 *recognition mechanism to human mental-retardation syndrome.* Nature structural
322 & molecular biology, 2011. **18**(7): p. 769-76.
- 323 28. Gibbons, R.J., et al., *Mutations in the chromatin-associated protein ATRX.* Human
324 mutation, 2008. **29**(6): p. 796-802.

29. Lipps, H.J. and D. Rhodes, *G-quadruplex structures: in vivo evidence and function*. Trends in cell biology, 2009. **19**(8): p. 414-22.
30. Rodriguez, R., et al., *Small-molecule-induced DNA damage identifies alternative DNA structures in human genes*. Nat Chem Biol, 2012. **8**(3): p. 301-10.
31. Paeschke, K., J.A. Capra, and V.A. Zakian, *DNA replication through G-quadruplex motifs is promoted by the Saccharomyces cerevisiae Pif1 DNA helicase*. Cell, 2011. **145**(5): p. 678-91.
32. Elsassner, S.J., et al., *DAXX envelops a histone H3.3-H4 dimer for H3.3-specific recognition*. Nature, 2012. **491**(7425): p. 560-5.
33. Nakatani, Y., et al., *Two distinct nucleosome assembly pathways: dependent or independent of DNA synthesis promoted by histone H3.1 and H3.3 complexes*. Cold Spring Harb Symp Quant Biol, 2004. **69**: p. 273-80.
34. Ray-Gallet, D., et al., *Dynamics of histone H3 deposition in vivo reveal a nucleosome gap-filling mechanism for H3.3 to maintain chromatin integrity*. Molecular cell, 2011. **44**(6): p. 928-41.
35. Ribeyre, C., et al., *The yeast Pif1 helicase prevents genomic instability caused by G-quadruplex-forming CEB1 sequences in vivo*. PLoS genetics, 2009. **5**(5): p. e1000475.
36. Grand, C.L., et al., *The cationic porphyrin TMPyP4 down-regulates c-MYC and human telomerase reverse transcriptase expression and inhibits tumor growth in vivo*. Mol Cancer Ther, 2002. **1**(8): p. 565-73.
37. Rizzo, A., et al., *Stabilization of quadruplex DNA perturbs telomere replication leading to the activation of an ATR-dependent ATM signaling pathway*. Nucleic acids research, 2009. **37**(16): p. 5353-64.

- 349 38. Huh, M.S., et al., *Compromised genomic integrity impedes muscle growth after Atrx*
350 *inactivation*. J Clin Invest, 2012. **122**(12): p. 4412-23.
- 351 39. Leung, J.W., et al., *Alpha thalassemia/mental retardation syndrome X-linked gene*
352 *product ATRX is required for proper replication restart and cellular resistance to*
353 *replication stress*. The Journal of biological chemistry, 2013.
- 354 40. Sarkies, P., et al., *FANCI coordinates two pathways that maintain epigenetic*
355 *stability at G-quadruplex DNA*. Nucleic acids research, 2012. **40**(4): p. 1485-98.
- 356 41. Sarkies, P., et al., *Epigenetic instability due to defective replication of structured*
357 *DNA*. Molecular cell, 2010. **40**(5): p. 703-13.
- 358 42. Branzei, D. and M. Foiani, *The checkpoint response to replication stress*. DNA
359 Repair (Amst), 2009. **8**(9): p. 1038-46.
- 360 43. Petermann, E. and T. Helleday, *Pathways of mammalian replication fork restart*.
361 Nat Rev Mol Cell Biol, 2010. **11**(10): p. 683-7.
- 362 44. Shanbhag, N.M., et al., *ATM-dependent chromatin changes silence*
363 *transcription in cis to DNA double-strand breaks*. Cell, 2010. **141**(6): p. 970-81.
- 364 45. Pflumm, M.F., *The role of DNA replication in chromosome condensation*. Bioessays,
365 2002. **24**(5): p. 411-8.
- 366 46. Shay, J.W. and S. Bacchetti, *A survey of telomerase activity in human cancer*. Eur J
367 Cancer, 1997. **33**(5): p. 787-91.
- 368 47. Blackburn, E.H., C.W. Greider, and J.W. Szostak, *Telomeres and telomerase: the*
369 *path from maize, Tetrahymena and yeast to human cancer and aging*. Nat Med,
370 2006. **12**(10): p. 1133-8.

48. Cesare, A.J. and R.R. Reddel, *Alternative lengthening of telomeres: models, mechanisms and implications*. Nat Rev Genet, 2010. **11**(5): p. 319-30.
49. Shay, J.W., R.R. Reddel, and W.E. Wright, *Cancer. Cancer and telomeres--an ALternative to telomerase*. Science, 2012. **336**(6087): p. 1388-90.
50. Sfeir, A., et al., *Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication*. Cell, 2009. **138**(1): p. 90-103.
51. Ratnakumar, K., et al., *ATRX-mediated chromatin association of histone variant macroH2A1 regulates alpha-globin expression*. Genes & development, 2012. **26**(5): p. 433-8.
52. Zhang, R., et al., *Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA*. Dev Cell, 2005. **8**(1): p. 19-30.

Figure legends

Figure 1. Recruitment of ATRX to chromatin. Schematic depicting binding of ATRX to histone H3 at heterochromatic sites through an interaction of the ADD domain with the histone H3 N-terminal tail, trimethylated at Lys9 and unmodified at Lys4. Recruitment is enhanced by a third interaction through HP1 that also recognises trimethylated Lys9 [23, 27]. ATRX also binds directly to G-quadruplex structured DNA (far right) [21]. Once recruited to its target sites ATRX, in combination with its interaction partner DAXX facilitate the deposition of the histone variant H3.3 [6, 8, 9, 32], which may maintain DNA in the B-form.

Figure 2. Stalling of DNA replication may lead to transcriptional silencing and aberrant telomeric recombination upon loss of ATRX function. The persistence of DNA secondary structures, particularly on the lagging strand, in the absence of functional ATRX may lead to replication fork stalling. Upon collapse of the fork a double strand break is generated which may result in the silencing of genes distal to the break site through RNF8 and RNF168 mediated ubiquitylation of H2A and the subsequent stalling of RNAPII [44]. Double strand breaks are a known trigger for homologous recombination mediated repair, which at telomeric sites may in turn trigger the ALT pathway in malignant cells lacking ATRX.



