

Specialized RSC: Substrate Specificities for a Conserved Chromatin Remodeler

Sarah J. Hainer* and Craig D. Kaplan*

The remodel the structure of chromatin (RSC) nucleosome remodeling complex is a conserved chromatin regulator with roles in chromatin organization, especially over nucleosome depleted regions therefore functioning in gene expression. Recent reports in *Saccharomyces cerevisiae* have identified specificities in RSC activity toward certain types of nucleosomes. RSC has now been shown to preferentially evict nucleosomes containing the histone variant H2A.Z in vitro. Furthermore, biochemical activities of distinct RSC complexes has been found to differ when their nucleosome substrate is partially unraveled. Mammalian BAF complexes, the homologs of yeast RSC and SWI/SNF complexes, are also linked to nucleosomes with H2A.Z, but this relationship may be complex and extent of conservation remains to be determined. The interplay of remodelers with specific nucleosome substrates and regulation of remodeler outcomes by nucleosome composition are tantalizing questions given the wave of structural data emerging for RSC and other SWI/SNF family remodelers.

their nucleosome substrates. What are the nucleosomal cues that elicit or alter remodeler activity to achieve different remodeling outcomes at different nucleosomes? How are remodelers attuned to deal with particular substrates or particular contexts? We discuss these issues with a primary focus on one recent publication linking RSC remodeling to nucleosomes containing the histone variant H2A.Z and a preprint demonstrating distinct biochemical activities of RSC variant complexes toward partially unwrapped nucleosomes. We then extend our discussion to an overview of mammalian SWI/SNF (SWItch/Sucose Non-Fermentable)-family remodelers (BRG1- or BRM-associated factors) and how we might think about their function and regulation by different nucleosome substrates.

1. Introduction

Assembly of DNA into chromatin forms the basis of a genome-wide platform to condense, protect, limit, and regulate interactions with genetic material. The primary unit of chromatin, the nucleosome, is an amazingly plastic platform upon which regulatory processes can operate. Many chromatin-interacting systems employ chromatin remodeling complexes as engines for modulating functional interactions with chromatin. Remodelers can remove nucleosomes by ejecting the histone octamer, slide nucleosomes by altering the position of the histone octamer relative to DNA sequence, or otherwise alter the composition of the histone octamer or accessibility of nucleosomal DNA. A number of intriguing mechanistic questions are open in regards to how chromatin remodelers are recruited and regulated by

2. RSC as a Paradigm for Chromatin Remodeling at Promoters

RSC was originally purified and cloned in yeast as a remodeling complex containing homologous subunits to the first discovered remodeler prototype, the SWI/SNF complex.^[1] Of interest, RSC is much more abundant, and in contrast to SWI/SNF, the majority of RSC components were found to be essential. Broadly, the current view of RSC function is that RSC is widely associated with yeast promoters containing defined nucleosome-free or depleted regions and tRNA genes.^[2-6] Earlier approaches to assess RSC function applied temperature-sensitive degron alleles to deplete the catalytic subunit Sth1 and required heat shock while chromatin structure changes were examined by tiling microarrays.^[5,7] More recent approaches have utilized auxin-inducible degrons or anchor away of Sth1, which do not require additional heat shock, and examined gene expression and nucleosome positioning with base-pair precision by deep sequencing.^[8,9]

These studies have found that RSC function maintains the width of nucleosome-free/depleted regions at a large class of promoters specified by positioned +1 and -1 nucleosomes, which themselves often incorporate the histone variant H2A.Z (Htz1 in yeast).^[10,11] In many cases, these promoters contain binding sites for transcription factors of a specific class (general regulatory factors such as Reb1, Abf1, and Rap1) thought to function in chromatin organization and potentially insulation by a number of mechanisms.^[7,12-15] When RSC is depleted,

Dr. S. J. Hainer, Dr. C. D. Kaplan
Department of Biological Sciences
University of Pittsburgh
Pittsburgh, PA 15260, USA
E-mail: sarah.hainer@pitt.edu; craig.kaplan@pitt.edu

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/bies.202000002>

© 2020 The Authors. *BioEssays* published by Wiley Periodicals LLC. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

DOI: 10.1002/bies.202000002

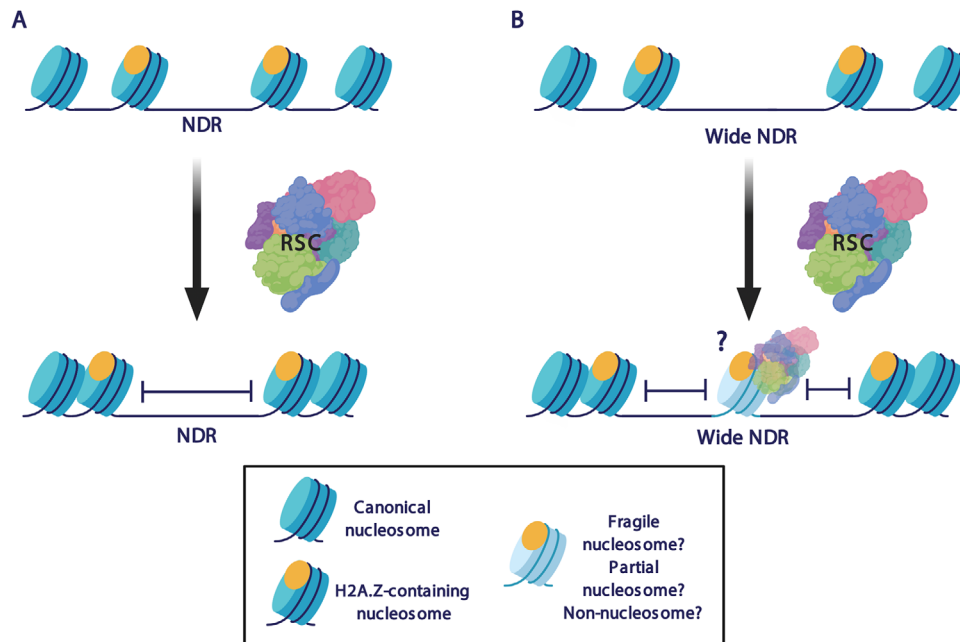


Figure 1. RSC function at H2A.Z flanking nucleosome depleted promoters in yeast. A) RSC acts on NDRs to open this region through remodeling of H2A.Z containing nucleosomes. B) Over wide NDRs, RSC restructures chromatin through remodeling of H2A.Z-containing nucleosomes and potential interaction with resident fragile nucleosomes.

nucleosomes encroach upon promoter nucleosome free regions (NFRs) with the consequence that a number of accessible transcription start sites (TSSs) are lost^[7–9,16] (Figure 1). Genetic and genomic studies suggest that RSC function on these nucleosomes reflects antagonism with additional remodelers^[8,16,17] and that these regions may be relatively nucleosome free in vitro due to energetic considerations, while in vivo RSC is required to maintain them.^[18] This being said, sequences enriched in these promoter regions are thought to promote RSC recruitment and potentially direct RSC activity directionally outward on the flanking nucleosomes.^[19–21]

3. RSC and H2A.Z: New Wrinkles to a Long-Term Relationship

RSC being discovered to function at NFR promoters in yeast puts RSC in proximity to the nucleosomes with the greatest enrichment of H2A.Z. Classic experiments from Hartley and Madhani indicated that RSC activity supporting the NFR was upstream of H2A.Z deposition, which required formation of the NFR prior to deposition of H2A.Z.^[7] A recent study suggests that H2A.Z is required for high occupancy of SWR1 (SWi2/snf2-Related) and INO80 (INOsitol requiring) complexes,^[22] the former required for H2A.Z deposition and the latter implicated in H2A.Z turnover (see Box 1).^[23] H2A.Z nucleosomes are found to be intrinsically less stable than canonical H2A-containing nucleosomes.^[24,25] INO80 is implicated in H2A.Z turnover because H2A.Z nucleosomes are more sensitive to INO80 remodeling relative to H2A-containing nucleosomes.^[26] These observations support the idea that nucleosome composition or properties

affect remodeler recruitment and/or remodeling outcomes. With these ideas in mind, recent work from Cakiroglu et al. is of special interest exploring the role of RSC remodeling H2A.Z-containing nucleosomes.^[27]

To ask if specific nucleosomes might be sensitive to RSC remodeling, Cakiroglu and colleagues took a novel and intriguing approach: purification of chromatin in the form of nucleosome associated material with the intent to maintain modifications, histone or histone variant composition, and the association of these nucleosomes with the DNA sequences they had occupied in vivo. These experiments extend the very elegant study by Lorch et al., where a purified chromatin ring of a single promoter was exposed to RSC and ATP, resulting in removal of promoter, but not genic, nucleosomes.^[21] In Cakiroglu et al., chromatin was solubilized to nucleosomes and associated material from chromatin by micrococcal nuclease (MNase), then purified en masse with a DEAE column and a sucrose gradient following DNA fragments, with final material obtained from fractions containing mononucleosome-sized DNA fragments. Chromatin surviving this purification was subjected to a remodeling assay by the addition of RSC and the histone chaperone, Nap1. Under these in vitro conditions, RSC is able to eject some or all histones because Nap1 acts as a histone acceptor.^[28–31] The authors hypothesized that a subset of nucleosomes might be sensitive to RSC activity and these nucleosomes might preferentially show octamer ejection by RSC in the presence of Nap1. Therefore, subsequent to RSC/Nap1 treatment, control and remodeled material was separated by native gel electrophoresis and DNA of differential mobility—that of free fragments or comigration with mononucleosomes—purified and subjected to Illumina sequencing. The different populations of DNA could then be mapped to specific genomic locations. DNA of

Box 1

Role of Non-SWI/SNF Family Nucleosome Remodelers in H2A.Z Regulation

While we have focused on the function of SWI/SNF family remodelers on H2A.Z-containing nucleosomes, we note that additional nucleosome remodelers act on H2A.Z-containing nucleosomes. SWR1 loads H2A.Z onto chromatin^[59] acting as a histone chaperone for the H2A.Z-H2B dimer.^[60,61] The histone acetyltransferase complex NuA4 is able to acetylate H2A.Z through the activity of the Esa1 subunit.^[62] Esa1 activity and the mammalian homolog of Esa1, Tip60, stimulate H2A.Z loading by H4 and H2A acetylation within the nucleosome as these marks recruit SWR1 complex.^[63] The mammalian homologs of SWR1 are p400 and SRCAP with SRCAP first shown to catalyze the incorporation of H2A.Z.^[64] Interestingly, mammalian p400 acts to-

gether with Tip60, representing a physical merge of the yeast NuA4 and SWR1 complexes to generate an apparent singular p400/Tip60 complex. However, a recent preprint from the Becker group demonstrates that *Drosophila* maintain the separation of SWR1 and NuA4 through alternative splice encoded isoforms of Domino (p400 homolog), where DOM-B-containing complex acts similarly to SWR1 to incorporate the *Drosophila* H2A.Z (H2A.V) and DOM-A-containing complex acts similarly to NuA4 to acetylate histone H4.^[65] As noted, Ino80 is able to preferentially exchange H2A.Z for H2A.^[26] The activities of these complexes on H2A.Z-containing nucleosomes are required for appropriate activity of these nucleosomes.

mononucleosomal size but with the mobility of free DNA was attributed to putative unstable nucleosomes. Genome positions showing reduced DNA occupancy corresponding to increased free DNA upon RSC/Nap1 treatment were attributed to putative nucleosome species sensitive to RSC activity. These experiments are inherently difficult and require correction for the putative instability of each occupied sequence based on comparison of multiple datasets; therefore, the authors focused only on the most RSC-sensitive nucleosomes. Highly RSC-sensitive DNA fragments (that have their mobility in native gels altered by RSC), consistent with RSC ejection of histone octamers, were found to be enriched for promoters, possibly coincident with either +1, -1, or potentially “fragile” nucleosomes (see below for discussion of fragile nucleosomes and RSC). This was in contrast to DNAs found free in the experiment without RSC treatment attributed to unstable nucleosomes.

The authors sought to identify other coincident attributes of these putative nucleosomes that might explain their sensitivity to RSC by comparing signal in their assay to signal from some classic ChIP-chip data on modified nucleosomes. We note a more stringent analysis could have employed more recent data (such as ref. [32]). Regardless, their analysis did not reveal association with histone modifications but did suggest potential association with H2A.Z nucleosomes. Given the promoter association of RSC-liberated DNA sequences, this was a provocative but sensical result. To test the relationship of RSC-sensitivity and H2A.Z, the authors repeated their *in vitro* remodeling of native purified chromatin from an *htz1Δ* strain. Excitingly, they found that the absence of H2A.Z appeared to alter the RSC-sensitivity of the previously highly RSC-sensitive nucleosomes. In an elegant orthogonal experiment, the authors directly assay RSC ejection of octamers from purified nucleosome arrays assembled with H2A or H2A.Z. Consistent with their prior result, RSC showed increased ability to eject octamers when H2A.Z was incorporated. Together, these results demonstrated an enhanced activity for RSC on H2A.Z-containing nucleosomes. Future experiments with RSC and H2A.Z containing nucleosomes (traditional mononucleosome or dinucleosome remodeling assays with and

without chaperone) may reveal additional properties of potential RSC specialization toward H2A.Z.

These results raise the question of how H2A.Z functionally interacts with RSC. There are a number of non-mutually exclusive possibilities. H2A.Z may facilitate RSC targeting of H2A.Z-containing nucleosomes, RSC may differentially remodel H2A.Z nucleosomes due to H2A.Z modulation of RSC function, or RSC may differentially remodel H2A.Z nucleosomes due to altered properties conferred to the nucleosome by H2A.Z. For example, H2A.Z nucleosomes have been described as less stable than H2A-containing and this has been interpreted as an explanation for displacement of H2A.Z-H2B dimers in response to remodeling by INO80.^[26] It is also possible that H2A.Z indirectly leads to an additional alteration of these nucleosomes that is not yet ascertained. However, H2A.Z deletion has subtle phenotypes in yeast compared to the essentiality of RSC and the large decreases in transcription by all three nuclear RNA polymerases that result from RSC depletion.^[5] Therefore, it does not seem that RSC targeting might be highly affected by loss of H2A.Z. RSC instead has been implicated in recognizing promoters directly either through DNA recognition of its own subunits or potential recruitment by DNA binding regulators.^[7,12,15,19,20,33] Another major open question is what does the RSC-sensitivity of H2A.Z nucleosomes reflect? A biophysical property of H2A.Z nucleosomes that is uncovered by remodeling in the presence of Nap1 only, or an activity that acts *in vivo*? As noted above, depletion of RSC results in encroachment of flanking nucleosomes on the NFR, a result consistent with the absence of RSC-mediated sliding or the absence of a RSC-mediated block to sliding by other remodelers, not necessarily the absence of ejection. Importantly, there have been no concerted studies of H2A.Z deposition in the absence of RSC in yeast. Limited experiments performed by Hartley et al. suggested that H2A.Z occupancy was reduced when Sth1 was targeted by a temperature-sensitive degron.^[7] To address these questions more directly may require a closer analysis of exactly which putative nucleosomes are affected on an individual promoter and nucleosome level, and comparison with perplexing data from a number of labs discussed immediately below.

4. RSC and “Fragile” Nucleosomes—An Enigmatic Species and an Enigmatic Relationship

Comparison of localization of putative nucleosomes hypersensitive to RSC to bulk nucleosome positions identified by MNase digestion appears to locate RSC-sensitive fragments within NFRs. Such positioning is reminiscent of a perplexing and difficult to understand series of contradictory observations in yeast chromatin. These observations stem from the inability to attribute a definitive molecular nature to partially MNase-resistant NFRs. These NFRs show signal when chromatin is lightly digested with MNase and mononucleosomal-sized bands are isolated. With more extensive digestion, they are greatly reduced. Initial interpretation was that this subset of NFRs, generally wider than normal NFRs, contained “fragile” nucleosomes of altered structure or sensitivity to digestion. A region of the *GAL1-10* UAS with similar properties was reported to be bound by RSC, histones, and specifically H2A.Z.^[34] Intriguingly, this region shows high signal in the Cakiroglu data, but for all fractions, both free DNA and mobility-shifted material with or without RSC remodeling. An early ChIP-chip study also observed H3 interaction within NFRs that showed no signal from heavy MNase digestion.^[35] Upon removal of RSC, a subset of putative fragile nucleosomes appeared stabilized;^[4,7,16] however, the major effect of RSC removal is apparent encroachment of flanking nucleosomes into the NFR. In the presence of RSC, these regions can show histone signal in some ChIP-seq assays.^[4,34] However, other studies employing a number of strategies using ChIP crosslinking or crosslinking plus light MNase digestion,^[36] chemical cleavage induced by specific H4 or H3 mutants,^[37,38] or ChIP-exo,^[32] have been unable to find positive association of tested histones with these regions. A further difficulty in examining these regions, for example, if they are fragile due to RSC action, is that upon removal of RSC, flanking nucleosomes encroach and likely invade territory of any putative fragile nucleosome. Recently, employing CUT&RUN for RSC followed by IP (CUT&RUN.ChIP), the Henikoff lab found RSC localization to wide NFRs, and in the immunoprecipitated material from the initial CUT&RUN liberated fragments, histones were present allowing further purification of fragments from the NFRs^[2] (Figure 1). Interestingly, ChIP-exo for RSC in the absence of heat shock shows RSC associating broadly with the genome but enriched over nucleosomes flanking NFRs, yet upon heat shock, RSC localization shifts to high enrichment within NFRs between flanking nucleosomes.^[3] These locations in the genome, regardless of their composition have a relationship with RSC that is intriguing.

5. RSC Function and Distinct Nucleosomal Substrates

The above question was raised regarding how remodelers act on specific substrates and how their activities may be tuned to specific nucleosomes. Increasingly, we have structural information on a wide range of remodelers, including a number of very recent RSC-nucleosome structures,^[39–41] a BAF-nucleosome structure,^[42] and a yeast SWI/SNF^[43] structure (see **Box 2**). There are a number of known or predicted DNA and protein interaction domains within RSC; for example, RSC contains eight bro-

modomains distributed among five subunits, implicated in modified histone interactions (two are in the mutually exclusive Rsc1 and Rsc2 paralogous subunits). The new structures will be incredibly valuable, but their histone interacting bromodomains and histone tails themselves are not generally observable in the structures. This being said, it is not well understood how all of these domains might function in RSC targeting or regulation. Remodelers can be simple single-subunit enzymes, or they can be massive and complex, like RSC and homologous complexes across eukaryotes. In many cases, including RSC, there are mutually exclusive components that are incorporated into distinct versions of each complex. A recent preprint from the Cairns lab sheds new light on a potential reason that mutually exclusive subunits might be utilized.^[44] In this case, RSC subunits Rsc1 and Rsc2 confer differing abilities to RSC for remodeling of partially unwrapped nucleosomes. Through elegant biochemical and genetic studies, Rsc1-containing RSC is found to much better tolerate unwrapping of the nucleosome for remodeling in vitro, while Rsc2-containing RSC does not. Supporting this biochemical difference, *rsc1Δ* cells are much more sensitive to a number of other genetic defects than *rsc2Δ*, such as mutations that promote entry–exit unwrapping, consistent with the Rsc1 complex (the only RSC present in *rsc2Δ* cells) being more robust than the Rsc2 complex (the only RSC present in *rsc1Δ* cells). Given the speculation that fragile nucleosomes could be remodeled, partially unwrapped, or potentially RSC bound altered nucleosome, differential activity of RSC complexes on unwrapped nucleosomes is provocative.

6. Mammalian Remodelers: Parallels between BAF and RSC

There are ≈30 nucleosome remodeling factor ATPases in mammalian cells, each fulfilling a distinct niche within the cell—often including both activating and repressing roles in gene transcription (reviewed in ref. [23,66,67]). Additional complexity emerges from the diversity in associated factors with these ATPases as we discuss below. Within the SWI/SNF family of remodelers, a specific BAF complex (PBAF, Polybromo-associated BAF) shares the closest similarity to yeast RSC, but as we now discuss, mammalian BAF complexes are diverse and complex.^[68,69] BAF complexes are approximately 2 MDa in size and contain up to 15 subunits, where many of these subunits are encoded by gene families and can therefore be replaced by their paralogues, akin to Rsc1 and Rsc2 in yeast. Core yeast SWI/SNF and RSC subunits have been conserved in mammalian complexes while a number of others have been added, increasing complexity in compositions greatly (reviewed in ref. [70,71]).

Mammalian BAF complexes are vast and often unique to cell type, raising questions about how else their functions might be tailored or specific. At least six cell type-specific BAF complexes have been identified, including those specific to cardiac cells,^[72,73] neural progenitors (npBAF^[74–76]), neural cells (nBAF^[75,77]), hematopoietic stem cells (hscBAF^[78]), and embryonic stem (ES) cells (esBAF^[79,80]). Other non-cell-type specific BAF complexes have been defined by subunit composition, including GLTSCR1-containing BAF (gBAF/ncBAF^[81–83]) and polybromo-containing BAF (PBAF^[84]), which are distinct

Box 2
Recent Cryo-EM Structures

Four recent yeast structures (three of RSC, one of SWI/SNF) and one of a mammalian BAF complex bound to the nucleosome provide intricate details regarding these interactions. Importantly, all three RSC-nucleosome structures demonstrate different aspects of this interaction, yet still reinforce the major interactions observed. The Cairns, Gao, and Chen groups found that the majority of the interaction with the nucleosome occurs through the ATPase Sth1, while Sfh1 interacts with the H2A-H2B acidic patch and histone H4 tail.^[39] Here the authors have poor resolution of the Sfh1 interaction, but additional biochemical data demonstrates the C-terminal tail of Sfh1 is required for this interaction. The orientation of these domains reveals directionality of DNA translocation. The Nogales group solved their RSC-nucleosome structure using a modified nucleosome (H3K4me3 and H3(K9/14/18)ac) for increased interaction with RSC.^[40] While this structure does not contain the ATPase domain of Sth1, they found major interactions between the nucleosome and RSC subunits Sth1, Rsc8, and Npl6. Finally, the Cramer group found that RSC can sandwich the nucleosome where the Sth1 SnAC domain (part of the ATPase module) and Sfh1 helix (part of an “arm” module) interact on opposite sides of the nucleosome to maintain the octamer while simultaneously translocating the surrounding DNA.^[41] The He group solved the structure of yeast SWI/SNF in complex with the nucleosome, where they observe interaction of Snf2 and Snf5 with the nucleosome and multiple additional subunits interacting with nucleosomal DNA.^[43] Finally, the Xu group solved the structure of human BAF with the nucleosome.^[42] Similar to the RSC structures, the authors reveal a direct interaction between the ATPase and

SMARCB1, a homolog of Sfh1/Snf5, with the nucleosome. Importantly, a number of putative nucleosome interacting regions (e.g., bromodomains and additional modules) appear to be flexible and have not been visualized so the current structures are only the beginning of next-level structural understanding of remodeler regulation by nucleosome interactions.

Of significance to this perspective, we note that all three RSC structures identify interaction between RSC and the acidic patch of H2A-H2B. Furthermore, the Snf5 and SMARCB1 subunits of SWI/SNF and BAF, respectively, also interact with the acidic patch. The acidic patch is a nexus for factor–nucleosome interactions (see ref. [45–56]). Importantly, H2A.Z has an extended acidic patch relative to H2A and three amino acid alterations, where N89, N94, and K95 from H2A are G92, D97, and S98 in H2A.Z^[57] (for review see ref. [58]). As proposed by Wagner et al. (Cramer group), it is possible the interaction between RSC subunit Sfh1 and the acidic patch may assist in substrate selection, specifically for H2A.Z due to the extended acidic patch.^[41] The Sfh1 helix forms a finger, enriched for basic arginine residues. Interestingly, the H2A.Z C-terminal tail also differs from the H2A C-terminal tail, by ten amino acids, and this region may serve as additional substrate recognition through additional RSC subunits. In addition, Ye et al. (Cairns, Gao, and Chen groups) elegantly demonstrate that the C-terminal tail of Sfh1 is necessary for nucleosome eviction on closed circular arrays, suggesting a requirement for this interaction to stabilize RSC association to the nucleosome and permit ejection.^[39] It will be interesting to determine structures of RSC or other remodelers with H2A.Z containing nucleosomes.

from the canonical BAF complex (cBAF). Importantly, the majority of these complexes can assemble with either ATPase (Brg1 or BRM), therefore, providing potential alternative functions for each cell-specific complex. This becomes especially important to consider in light of the recent preprint described above, ascribing alternative functions for Rsc1 and Rsc2. The different combinatorial assemblies of the BAF complex have been reviewed extensively elsewhere.^[71,85–87] These assemblies ensure complex specificity and allow the BAF complex to perform elaborate regulation within mammalian genomes.

While the majority of the subunits that are different between cell-type specific complexes are members of the same family (for example, use of BAF47A/B/C) and therefore broadly contain the same domain architecture, some notable exceptions exist. The most newly described BAF complex, GBAF or ncBAF, does not contain a BAF250/BAF200 (ARID1/2) subunit.^[82] ARID1A/B and ARID2 subunits are the most frequently disrupted subunits in a range of cancers and are important for complex recruitment to chromatin (reviewed in ref. [86,88]). GLTSCR1/1L replace ARID1/2 in GBAF and these proteins contain a GLTSCR domain

which is required for ncBAF interaction. Other intriguing differences between noncanonical and canonical BAF complexes include the use of BRD7 or BRD9 in PBAF or GBAF complexes, respectively.^[82–84,89] BRD7/9 are members of the bromodomain-containing protein family that bind to acetylated histone proteins. Therefore, inclusion of these subunits may permit alternative recruitment to chromatin or regulation/interaction with distinct nucleosome substrates for PBAF and GBAF. Finally, focusing on the ATPase of BAF complexes (Brg1 and BRM), the majority of BAF complexes can assemble with either Brg1 or BRM. However, esBAF and hscBAF are notable exceptions, where esBAF only contains Brg1, as BRM is not expressed in ES cells and hscBAF contains only BRM.^[78,79]

Similar to yeast RSC, BAF localizes to many promoter regions through mammalian genomes (reviewed in ref. [87]). In addition, BAF localizes to gene distal regulatory regions within mammalian genomes, such as enhancers.^[90] Both promoter regions (+1 and –1 nucleosomes) and enhancer regions (both flanking nucleosomes) are enriched for H2A.Z-containing nucleosomes and potential fragile nucleosomes or subnucleosomal species, as

with promoter regions within the yeast genome.^[24,91–96] At these locations, BAF performs similar molecular functions as RSC, to open the NDR and regulate transcription. Just as in yeast, H2A.Z is incorporated into nucleosomes through SWR1-related nucleosome remodeling ATPases, p400 and SRCAP. Surprisingly, one of our groups previously found that upon depletion of the catalytic subunit of esBAF (Brg1), H2A.Z occupancy is greatly reduced in ES cells,^[97] consistent with a role for Brg1 in enhancing H2A.Z localization or incorporation. Furthermore, it was found that esBAF plays a role in subnucleosome formation where depletion of Brg1 resulted in increased subnucleosome footprints, often at sites of H2A.Z-containing nucleosomes. These results suggested a role for esBAF in promoting nucleosome occupancy by stabilizing H2A.Z-containing nucleosomes or promoting H2A.Z deposition. In light of the above described results, these data suggest a role for esBAF in remodeling H2A.Z-containing nucleosomes to promote or preserve mature nucleosomes.

7. Conclusions

Nucleosome remodeling complexes are elegant molecular machines that are required for appropriate chromatin reorganization and transcription. The multiple mechanisms through which RSC and BAF carry out their biochemical activity are still being assigned. The two described papers provide new insight into RSC mechanisms and help to develop further models for BAF complexes.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

chromatin remodeler, H2AZ, histone variant, nucleosome, RSC

Received: January 8, 2020

Revised: March 11, 2020

Published online:

- [1] B. R. Cairns, Y. Lorch, Y. Li, M. Zhang, L. Lacomis, H. Erdjument-Bromage, P. Tempst, J. Du, B. Laurent, R. D. Kornberg, *Cell* **1996**, *87*, 1249.
- [2] S. Brahma, S. Henikoff, *Mol. Cell* **2019**, *73*, 238.
- [3] V. Vinayachandran, R. Reja, M. J. Rossi, B. Park, L. Rieber, C. Mittal, S. Mahony, B. F. Pugh, *Genome Res.* **2018**, *28*, 357.
- [4] S. Kubik, M. J. Bruzzone, P. Jacquet, J. L. Falcone, J. Rougemont, D. Shore, *Mol. Cell* **2015**, *60*, 422.
- [5] T. J. Parnell, J. T. Huff, B. R. Cairns, *EMBO J.* **2008**, *27*, 100.
- [6] H. H. Ng, F. Robert, R. A. Young, K. Struhl, *Genes Dev.* **2002**, *16*, 806.
- [7] P. D. Hartley, H. D. Madhani, *Cell* **2009**, *137*, 445.
- [8] S. Kubik, M. J. Bruzzone, D. Challal, R. Dreos, S. Mattarocci, P. Bucher, D. Libri, D. Shore, *Nat. Struct. Mol. Biol.* **2019**, *26*, 744.
- [9] A. Klein-Brill, D. Joseph-Strauss, A. Appleboim, N. Friedman, *Cell Rep.* **2019**, *26*, 279.
- [10] R. M. Raisner, P. D. Hartley, M. D. Meneghini, M. Z. Bao, C. L. Liu, S. L. Schreiber, O. J. Rando, H. D. Madhani, *Cell* **2005**, *123*, 233.
- [11] I. Albert, T. N. Mavrich, L. P. Tomsho, J. Qi, S. J. Zanton, S. C. Schuster, B. F. Pugh, *Nature* **2007**, *446*, 572.
- [12] M. J. Rossi, W. K. M. Lai, B. F. Pugh, *Genome Res.* **2018**, *28*, 497.
- [13] D. Challal, M. Barucco, S. Kubik, F. Feuerbach, T. Candelli, H. Geofroy, C. Benaksas, D. Shore, D. Libri, *Mol. Cell* **2018**, *72*, 955.
- [14] H. S. Rhee, B. F. Pugh, *Cell* **2011**, *147*, 1408.
- [15] R. T. Koerber, H. S. Rhee, C. Jiang, B. F. Pugh, *Mol. Cell* **2009**, *35*, 889.
- [16] D. Ganguli, R. V. Chereji, J. R. Iben, H. A. Cole, D. J. Clark, *Genome Res.* **2014**, *24*, 1637.
- [17] T. J. Parnell, A. Schlichter, B. G. Wilson, B. R. Cairns, *eLife* **2015**, *4*, e06073.
- [18] N. Krientein, M. Wal, S. Watanabe, B. Park, C. L. Peterson, B. F. Pugh, P. Korber, *Cell* **2016**, *167*, 709.
- [19] C. G. de Boer, T. R. Hughes, *PLoS One* **2014**, *9*, e110479.
- [20] G. Badis, E. T. Chan, H. van Bakel, L. Pena-Castillo, D. Tillo, K. Tsui, C. D. Carlson, A. J. Gossett, M. J. Hasiuff, C. L. Warren, M. Gebbia, S. Talukder, A. Yang, S. Mnaimneh, D. Terterov, D. Coburn, A. Li Yeo, Z. X. Yeo, N. D. Clarke, J. D. Lieb, A. Z. Ansari, C. Nislow, T. R. Hughes, *Mol. Cell* **2008**, *32*, 878.
- [21] Y. Lorch, J. Griesenbeck, H. Boeger, B. Maier-Davis, R. D. Kornberg, *Nat. Struct. Mol. Biol.* **2011**, *18*, 881.
- [22] D. N. Bagchi, A. M. Battenhouse, D. Park, V. R. Iyer, *Nucleic Acids Res.* **2019**, *48*, 157.
- [23] C. R. Clapier, J. Iwasa, B. R. Cairns, C. L. Peterson, *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 407.
- [24] C. Jin, G. Felsenfeld, *Genes Dev.* **2007**, *21*, 1519.
- [25] M. Marques, L. Laflamme, A. L. Gervais, L. Gaudreau, *Epigenetics* **2010**, *5*, 267.
- [26] S. Brahma, M. I. Udugama, J. Kim, A. Hada, S. K. Bhardwaj, S. G. Hailu, T. H. Lee, B. Bartholomew, *Nat. Commun.* **2017**, *8*, 15616.
- [27] A. Cakiroglu, C. R. Clapier, A. H. Ehrensberger, E. Darbo, B. R. Cairns, N. M. Luscombe, J. Q. Svejstrup, *Genome Res.* **2019**, *29*, 988.
- [28] K. W. Hsu, S. Y. Chow, B. Y. Su, Y. H. Lu, C. J. Chen, W. L. Chen, M. Y. Cheng, H. F. Fan, *BBA - Gene Regul. Mech.* **2019**, *1862*, 129.
- [29] R. Prasad, S. D'Arcy, A. Hada, K. Luger, B. Bartholomew, *Mol Cell Biol.* **2016**, *36*, 2262.
- [30] Y. Lorch, B. Maier-Davis, R. D. Kornberg, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3090.
- [31] B. G. Kuryan, J. Kim, N. N. Tran, S. R. Lombardo, S. Venkatesh, J. L. Workman, M. Carey, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 1931.
- [32] H. S. Rhee, A. R. Bataille, L. Zhang, B. F. Pugh, *Cell* **2014**, *159*, 1377.
- [33] M. L. Angus-Hill, A. Schlichter, D. Roberts, H. Erdjument-Bromage, P. Tempst, B. R. Cairns, *Mol. Cell* **2001**, *7*, 741.
- [34] M. Floer, X. Wang, V. Prabhu, G. Berrozpe, S. Narayan, D. Spagna, D. Alvarez, J. Kendall, A. Krasnitz, A. Stepansky, J. Hicks, G. O. Bryant, M. Ptashne, *Cell* **2010**, *141*, 407.
- [35] A. Rufiange, P. E. Jacques, W. Bhat, F. Robert, A. Nourani, *Mol. Cell* **2007**, *27*, 393.
- [36] R. V. Chereji, J. Ocampo, D. J. Clark, *Mol. Cell* **2017**, *65*, 565.
- [37] K. Brogaard, L. Xi, J. P. Wang, J. Widom, *Nature* **2012**, *486*, 496.
- [38] S. Ramachandran, G. E. Zentner, S. Henikoff, *Genome Res.* **2015**, *25*, 381.
- [39] Y. Ye, H. Wu, K. Chen, C. R. Clapier, N. Verma, W. Zhang, H. Deng, B. R. Cairns, N. Gao, Z. Chen, *Science* **2019**, *366*, 838.
- [40] A. B. Patel, C. M. Moore, B. J. Greber, J. Luo, S. A. Zukin, J. Ranish, E. Nogales, *eLife* **2019**, *8*, e54449.
- [41] F. R. Wagner, C. Dienemann, H. Wang, A. Stützer, D. Tegunov, H. Urlaub, P. Cramer, *Nature* **2020**, *579*, 448.
- [42] S. He, Z. Wu, Y. Tian, Z. Yu, J. Yu, X. Wang, J. Li, B. Liu, Y. Xu, *Science* **2020**, *367*, 875.
- [43] Y. Han, A. A. Reyes, S. Malik, Y. He, *Nature* **2020**, *579*, 452.
- [44] A. Schlichter, M. M. Kasten, T. J. Parnell, B. R. Cairns, *bioRxiv* **2019**.

- [45] H. T. Dao, B. E. Dul, G. P. Dann, G. P. Liszczak, T. W. Muir, *Nat. Chem. Biol.* **2020**, *16*, 134.
- [46] A. M. Valencia, C. K. Collings, H. T. Dao, R. St Pierre, Y. C. Cheng, J. Huang, Z. Y. Sun, H. S. Seo, N. Mashtalir, D. E. Comstock, O. Bolonduro, N. E. Vangos, Z. C. Yeoh, M. K. Dornon, C. Hermawan, L. Barrett, S. Dhe-Paganon, C. J. Woolf, T. W. Muir, C. Kadoch, *Cell* **2019**, *179*, 1342.
- [47] C. E. Cucinotta, A. E. Hildreth, B. M. McShane, M. K. Shirra, K. M. Arndt, *Nucleic Acids Res.* **2019**, *47*, 8410.
- [48] N. Gamarra, S. L. Johnson, M. J. Trnka, A. L. Burlingame, G. J. Narlikar, *eLife* **2018**, *7*, e35322.
- [49] A. J. Hodges, L. M. Gloss, J. J. Wyrick, *Genetics* **2017**, *206*, 1339.
- [50] G. T. Debelouchina, K. Gerecht, T. W. Muir, *Nat. Chem. Biol.* **2017**, *13*, 105.
- [51] Q. Chen, R. Yang, N. Korolev, C. F. Liu, L. Nordenskiold, *J Mol Biol.* **2017**, *429*.
- [52] C. E. Cucinotta, A. N. Young, K. M. Klucsevsek, K. M. Arndt, *PLoS Genet.* **2015**, *11*, e1005420.
- [53] F. Mattioli, M. Uckelmann, D. D. Sahtoe, W. J. van Dijk, T. K. Sixma, *Nat. Commun.* **2014**, *5*, 3291.
- [54] J. W. Leung, P. Agarwal, M. D. Canny, F. Gong, A. D. Robison, I. J. Finkelstein, D. Durocher, K. M. Miller, *PLoS Genet.* **2014**, *10*, e1004178.
- [55] V. Subramanian, A. Mazumder, L. E. Surface, V. L. Butty, P. A. Fields, A. Alwan, L. Torrey, K. K. Thai, S. S. Levine, M. Bathe, L. A. Boyer, *PLoS Genet.* **2013**, *9*, e1003725.
- [56] A. A. Kalashnikova, M. E. Porter-Goff, U. M. Muthurajan, K. Luger, J. C. Hansen, *J. R. Soc., Interface.* **2013**, *10*, 20121022.
- [57] R. K. Suto, M. J. Clarkson, D. J. Tremethick, K. Luger, *Nat. Struct. Biol.* **2000**, *7*, 1121.
- [58] C. Bonisch, S. B. Hake, *Nucleic Acids Res.* **2012**, *40*, 10719.
- [59] G. Mizuguchi, X. Shen, J. Landry, W. H. Wu, S. Sen, C. Wu, *Science* **2004**, *303*, 343.
- [60] Z. Mao, L. Pan, W. Wang, J. Sun, S. Shan, Q. Dong, X. Liang, L. Dai, X. Ding, S. Chen, Z. Zhang, B. Zhu, Z. Zhou, *Cell Res.* **2014**, *24*, 389.
- [61] J. Hong, H. Feng, F. Wang, A. Ranjan, J. Chen, J. Jiang, R. Ghirlando, T. S. Xiao, C. Wu, Y. Bai, *Mol. Cell* **2014**, *53*, 498.
- [62] M. C. Keogh, T. A. Mennella, C. Sawa, S. Berthelet, N. J. Krogan, A. Wolek, V. Podolny, L. R. Carpenter, J. F. Greenblatt, K. Baetz, S. Buratowski, *Genes Dev.* **2006**, *20*, 660.
- [63] M. Altaf, A. Auger, J. Monnet-Saksouk, J. Brodeur, S. Piquet, M. Cramet, N. Bouchard, N. Lacoste, R. T. Utey, L. Gaudreau, J. Cote, *J. Biol. Chem.* **2010**, *285*, 15966.
- [64] D. D. Ruhl, J. Jin, Y. Cai, S. Swanson, L. Florens, M. P. Washburn, R. C. Conaway, J. W. Conaway, J. C. Chrivia, *Biochemistry* **2006**, *45*, 5671.
- [65] A. Scacchetti, T. Schauer, A. Reim, Z. Apostolou, A. C. Sparr, S. Krause, P. Heun, M. Wierer, P. B. Becker, *bioRxiv* **2020**.
- [66] S. K. Hota, B. G. Bruneau, *Development* **2016**, *143*, 2882.
- [67] P. B. Becker, J. L. Workman, *Cold Spring Harbor Perspect. Biol.* **2013**, *5*, a017905.
- [68] W. Wang, T. Chi, Y. Xue, S. Zhou, A. Kuo, G. R. Crabtree, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 492.
- [69] Y. Xue, J. C. Canman, C. S. Lee, Z. Nie, D. Yang, G. T. Moreno, M. K. Young, E. D. Salmon, W. Wang, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13015.
- [70] L. Ho, G. R. Crabtree, *Nature* **2010**, *463*, 474.
- [71] A. Alfert, N. Moreno, K. Kerl, *Epigenetics Chromatin* **2019**, *12*, 19.
- [72] H. Lickert, J. K. Takeuchi, I. Von Both, J. R. Walls, F. McAuliffe, S. L. Adamson, R. M. Henkelman, J. L. Wrana, J. Rossant, B. G. Bruneau, *Nature* **2004**, *432*, 107.
- [73] J. K. Takeuchi, B. G. Bruneau, *Nature* **2009**, *459*, 708.
- [74] J. Lessard, J. I. Wu, J. A. Ranish, M. Wan, M. M. Winslow, B. T. Staahl, H. Wu, R. Aebersold, I. A. Graef, G. R. Crabtree, *Neuron* **2007**, *55*, 201.
- [75] J. I. Wu, J. Lessard, I. A. Olave, Z. Qiu, A. Ghosh, I. A. Graef, G. R. Crabtree, *Neuron* **2007**, *56*, 94.
- [76] D. A. Lamba, S. Hayes, M. O. Karl, T. Reh, *Dev. Dyn.* **2008**, *237*, 3016.
- [77] I. Olave, W. Wang, Y. Xue, A. Kuo, G. R. Crabtree, *Genes Dev.* **2002**, *16*, 2509.
- [78] M. Buscarlet, V. Krasteva, L. Ho, C. Simon, J. Hebert, B. Wilhelm, G. R. Crabtree, G. Sauvageau, P. Thibault, J. A. Lessard, *Blood* **2014**, *123*, 1720.
- [79] L. Ho, R. Jothi, J. L. Ronan, K. Cui, K. Zhao, G. R. Crabtree, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5187.
- [80] L. Ho, J. L. Ronan, J. Wu, B. T. Staahl, L. Chen, A. Kuo, J. Lessard, A. I. Nesvizhskii, J. Ranish, G. R. Crabtree, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 5181.
- [81] E. Middeljans, X. Wan, P. W. Jansen, V. Sharma, H. G. Stunnenberg, C. Logie, *PLoS One* **2012**, *7*, e33834.
- [82] A. Alpsy, E. C. Dykhuizen, *J. Biol. Chem.* **2018**, *293*, 3892.
- [83] J. Gatchalian, S. Malik, J. Ho, D. S. Lee, T. W. R. Kelso, M. N. Shokhirev, J. R. Dixon, D. C. Hargreaves, *Nat. Commun.* **2018**, *9*, 5139.
- [84] W. Wang, J. Cote, Y. Xue, S. Zhou, P. A. Khavari, S. R. Biggar, C. Muchardt, G. V. Kalpana, S. P. Goff, M. Yaniv, J. L. Workman, G. R. Crabtree, *EMBO J.* **1996**, *15*, 5370.
- [85] R. Mathur, C. W. M. Roberts, *Annu. Rev. Canc. Biol.* **2018**, *2*, 413.
- [86] C. Hodges, J. G. Kirkland, G. R. Crabtree, *Cold Spring Harbor Perspect. Med.* **2016**, *6*, a026930.
- [87] P. J. Ho, S. M. Lloyd, X. Bao, *Development* **2019**, *146*, dev178780.
- [88] C. Kadoch, G. R. Crabtree, *Sci. Adv.* **2015**, *1*, e1500447.
- [89] M. D. Kaeser, A. Aslanian, M. Q. Dong, J. R. Yates, 3rd, B. M. Emerson, *J. Biol. Chem.* **2008**, *283*, 32254.
- [90] A. Local, H. Huang, C. P. Albuquerque, N. Singh, A. Y. Lee, W. Wang, C. Wang, J. E. Hsia, A. K. Shiau, K. Ge, K. D. Corbett, D. Wang, H. Zhou, B. Ren, *Nat. Genet.* **2018**, *50*, 73.
- [91] C. Jin, C. Zang, G. Wei, K. Cui, W. Peng, K. Zhao, G. Felsenfeld, *Nat. Genet.* **2009**, *41*, 941.
- [92] J. Ernst, P. Kheradpour, T. S. Mikkelsen, N. Shores, L. D. Ward, C. B. Epstein, X. Zhang, L. Wang, R. Issner, M. Coyne, M. Ku, T. Durham, M. Kellis, B. E. Bernstein, *Nature* **2011**, *473*, 43.
- [93] M. Ku, J. D. Jaffe, R. P. Koche, E. Rheinbay, M. Endoh, H. Koseki, S. A. Carr, B. E. Bernstein, *Genome Biol.* **2012**, *13*, R85.
- [94] G. Hu, K. Cui, D. Northrup, C. Liu, C. Wang, Q. Tang, K. Ge, D. Levens, C. Crane-Robinson, K. Zhao, *Cell Stem Cell* **2013**, *12*, 180.
- [95] S. Henikoff, J. G. Henikoff, A. Sakai, G. B. Loeb, K. Ahmad, *Genome Res.* **2009**, *19*, 460.
- [96] A. D. Goldberg, L. A. Banaszynski, K. M. Noh, P. W. Lewis, S. J. El-saesser, S. Stadler, S. Dewell, M. Law, X. Guo, X. Li, D. Wen, A. Chappier, R. C. DeKaveler, J. C. Miller, Y. L. Lee, E. A. Boydston, M. C. Holmes, P. D. Gregory, J. M. Greal, S. Rafii, C. Yang, P. J. Scambler, D. Garrick, R. J. Gibbons, D. R. Higgs, I. M. Cristea, F. D. Urnov, D. Zheng, C. D. Allis, *Cell* **2010**, *140*, 678.
- [97] S. J. Hainer, T. G. Fazio, *Cell Rep.* **2015**, *13*, 61.