Title

Chromatin openness requires continuous SWI/SNF activity

Journal

Nature Genetics, 53(3)

ISSN

1061-4036

Author

Hargreaves, Diana C

Publication Date

2021-03-01

DOI

10.1038/s41588-021-00781-7

Peer reviewed

HHS Public Access

Author manuscript

Nat Genet. Author manuscript; available in PMC 2021 September 21.

Published in final edited form as:

Nat Genet. 2021 March ; 53(3): 263–264. doi:10.1038/s41588-021-00781-7.

Chromatin openness requires continuous SWI/SNF activity

Diana C. Hargreaves

Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA.

Abstract

Chromatin structure and specifically sites of open or accessible chromatin regulate transcription factor binding to determine cell type-specific gene expression. Two new studies find that there is a constant requirement for SWI/SNF complex remodeling to maintain open chromatin using chemical and genetic methods that acutely inhibit or degrade the BRG1/BRM ATPase subunits, leading to loss in transcription factor binding and altered gene expression.

Main

Mammalian SWI/SNF complexes use ATP that is hydrolyzed by a core ATPase, BRG1 (SMARCA4) or BRM (SMARCA2), to generate chromatin accessibility. Previous studies have utilized conditional knockout/knockdown to show that BRG1 is required to maintain chromatin accessibility at SWI/SNF complex target sites[1–4]. However, due to the slow decay in BRG1 protein in these systems (~72–96 hours), it was unclear whether BRG1 is continuously required to generate open chromatin, or only at defined cellular transitions, e.g. following DNA replication. Acute depletion of yeast SWI2 results in rapid changes in chromatin accessibility[5,6], but the dependence on cell cycle is undetermined. Thus, it is unclear how dynamic the generation of accessible chromatin is and what the impact of this process on transcription factor binding and gene expression is.

Dynamic remodeling

To address the requirement for chromatin remodeling in acute kinetic resolution, Iurlaro et al.[7] and Schick et al.[8] took complementary approaches to rapidly inactivate BRG1, using ATAC-seq to read out changes in chromatin accessibility. Iurlaro et al. used an ATPase inhibitor of BRG1/BRM, BRM014[9], in mouse embryonic stem cells (ESCs), which exclusively express BRG1. Schick et al. developed a genetic approach by engineering the HAP1 cell line to express a BRG1 dTAG fusion protein ($SMARCA4^{dTAG}$)[10]. Treatment with dTAG47 resulted in ~90% BRG1 degradation in 2 hours and complete degradation by 6 hours. Schick et al. additionally compared their results with dTAG47 to treatment with BRM014 or ACBI1, a chemical degrader of BRG1/BRM[11]. In all cases, the authors found that inhibition or degradation of BRG1/BRM resulted in extremely rapid changes in chromatin accessibility, primarily losses. In the case of BRM014 treatment, these changes

dhargreaves@salk.edu .

could be observed in as little as 5–10 minutes, while dTAG47 and ACBI1 degrader treatments resulted in changes as early as 6 and 24 hours, respectively.

Collectively, these data point to a dynamic process involving continuous generation of open chromatin by SWI/SNF complexes, and argue against a stable state that requires chromatin remodeling only at specific stages of the cell cycle. The use of BRM014 inhibitor suggests that ATPase activity of BRG1/BRM is required for this process; however, understanding how BRM014 affects other aspects of SWI/SNF complex biology such as chromatin binding is necessary to conclude this definitively. Additionally, while the specificity of BRM014 for BRG1/BRM is a potential caveat, the strong correlation between accessibility changes in BRM014-treated and BRG1 knockout cells, and not cells deficient in subunits of the INO80 and ISWI chromatin remodelers, argues for the on-target activity of BRM014. In this regard, the genetic experiments from Schick et al. provide a nice complementary study; however, it should be noted that dTAG introduction into the *SMARCA4* locus results in a ~50% decrease in endogenous BRG1 levels at steady state. This reduction results in baseline changes in chromatin accessibility and gene expression between the $SMARCA4^{dTAG}$ and parent line, and has the potential to cause long-term adaptations for example in the composition of SWI/SNF complexes or dependence on BRG1 that could lead to an underestimation of the full range of BRG1 activity. Nevertheless, the authors observe a continuous requirement for BRG1 for maintaining chromatin accessibility and a strong correlation between accessibility changes following BRG1 degradation and BRM014 inhibition in HAP1 cells.

Remodeling for transcription

Given the dependence on BRG1 for maintaining chromatin accessibility, both studies explored the sensitivity of epigenetic and transcriptional features to acute disruption of BRG1/BRM. The authors distinguished sites that lost accessibility quickly versus slowly following drug treatment. Enhancers were enriched in both classes, with fast responding sites having H3K4 mono- and di-methylation, and slow responding sites also having H3K27 acetylation (H3K27ac), a mark of active promoters and enhancers. Schick et al. further found that acute degradation of BRM in BRG1 KO cells reduced accessibility at super-enhancers, which are otherwise unaffected by loss of BRG1 alone, similar to what we observed under steady state conditions for ARID1A/ARID1B[12]. They suggest that the dependence on SWI/SNF complexes is correlated with H3K27ac levels at enhancers, with $H3K27ac^{-}$ being most sensitive, $H3K27ac^{+}$ responding more slowly, and $H3K27ac^{hi}$ super-enhancers being the most resistant. A number of things could contribute to this hierarchy, such as the density or activity of positive regulators at active and super-enhancers, which could buffer the effects of BRG1 loss or more effectively recruit limiting amounts of SWI/SNF complexes, as well as the absence of counteracting remodelers that position nucleosomes in the absence of BRG1[6].

Fast responding sites were enriched for OCT4/SOX2/NANOG binding in mouse ESCs, while REST was enriched at slow responding sites. By ChIP-seq, Iurlaro et al. found that OCT4 binding was completely lost within 30 minutes of BRM014 addition, while REST binding was unaffected in the first 2 hours, but reduced by 24 hours. Thus, the kinetics

of decline in transcription factor binding were correlated with fast versus slow loss in chromatin accessibility. In a similar way, changes in transcription and accessibility were well correlated with respect to directionality and rate of change. This trend was also observed by Schick et al., although the number of significant gene expression changes following dTAG47 or BRM014 treatment in HAP1 cells was minimal, perhaps reflecting cell type-specific differences between mouse ESCs and HAP1 cells.

These data argue that cell type-specific gene expression is maintained by the continuous establishment of accessible chromatin and remodeler-assisted transcription factor binding. These data are remarkable particularly for pioneer factors OCT4 and SOX2, which despite the ability to bind their motifs on nucleosome-bound DNA[13,14], require the constant action of BRG1 for binding in vivo. Further, they suggest that cell-intrinsic mechanisms, rather than cellular memory in the form of inherited epigenetic modifications, are sufficient to establish the accessible chromatin landscape. This is illustrated nicely by the rapid reestablishment of accessibility, reduced nucleosome occupancy and transcription upon BRM014 wash-out. The dynamic nature of chromatin accessibility suggests that this may be one process by which cells respond quickly to developmental and environmental signals to establish new gene expression. Additionally, it may provide a mechanism by which SWI/SNF subunit switching during differentiation[15,16] results in changes in complex targeting that rapidly influence the landscape of accessible chromatin and the utilization of new regulatory elements to drive lineage-specific transcriptional programs. Future experiments using time-resolved approaches will help unravel the mechanistic relationship between chromatin remodeling, transcription factor binding, and transcription.

References

1. Ho L. et al.esBAF facilitates pluripotency by conditioning the genome for LIF/STAT3 signalling and by regulating polycomb function. Nat Cell Biol13, 903–913, doi:10.1038/ncb2285 (2011). [PubMed: 21785422]
2. Miller ELet al.TOP2 synergizes with BAF chromatin remodeling for both resolution and formation of facultative heterochromatin. Nat Struct Mol Biol24, 344–352, doi:10.1038/nsmb.3384 (2017). [PubMed: 28250416]
3. King HW & Klose RJ The pioneer factor OCT4 requires the chromatin remodeller BRG1 to support gene regulatory element function in mouse embryonic stem cells. eLife 6, doi:10.7554/eLife.22631 (2017).
4. Bao X. et al.A novel ATAC-seq approach reveals lineage-specific reinforcement of the open chromatin landscape via cooperation between BAF and p63. Genome Biol16, 284, doi:10.1186/s13059-015-0840-9 (2015). [PubMed: 26683334]
5. Biggar SR & Crabtree GR Continuous and widespread roles for the Swi-Snf complex in transcription. The EMBO journal 18, 2254–2264, doi:10.1093/emboj/18.8.2254 (1999). [PubMed: 10205178]
6. Kubik S. et al.Opposing chromatin remodelers control transcription initiation frequency and start site selection. Nat Struct Mol Biol26, 744–754, doi:10.1038/s41594-019-0273-3 (2019). [PubMed: 31384063]
7. Iurlaro M, Stadler MB, Masoni F, Jagani Z, Galli GG, Schubeler D. Mammalian SWI/SNF continuously restores local accessibility to chromatin. Nat Genet [NGLE54362R3 Schübeler] (2020).
8. Schick S, Grosche S, Kohl KE, Drpic D, Jaeger MG, Marella N, Imrichova H, Lin J-MG, Hofstatter G, Schuster M, Rendeiro AF, Koren A, Petronczki M, Bock C, Muller AC, Winter GE, Kubicek

S. Acute BAF perturbation causes immediate changes in chromatin accessibility. Nat Genet [NG-LE54374R4 Kubicek] (2020).

9. Papillon JPNet al.Discovery of Orally Active Inhibitors of Brahma Homolog (BRM)/SMARCA2 ATPase Activity for the Treatment of Brahma Related Gene 1 (BRG1)/SMARCA4-Mutant Cancers. J Med Chem61, 10155–10172, doi:10.1021/acs.jmedchem.8b01318 (2018). [PubMed: 30339381]
10. Nabet B. et al.The dTAG system for immediate and target-specific protein degradation. Nat Chem Biol14, 431–441, doi:10.1038/s41589-018-0021-8 (2018). [PubMed: 29581585]
11. Farnaby W. et al.BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. Nat Chem Biol15, 672–680, doi:10.1038/s41589-019-0294-6 (2019). [PubMed: 31178587]
12. Kelso TWRet al.Chromatin accessibility underlies synthetic lethality of SWI/SNF subunits in ARID1A-mutant cancers. eLife6, doi:10.7554/eLife.30506 (2017).
13. Soufi A. et al.Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate reprogramming. Cell161, 555–568, doi:10.1016/j.cell.2015.03.017 (2015). [PubMed: 25892221]
14. Michael AKet al.Mechanisms of OCT4-SOX2 motif readout on nucleosomes. Science368, 1460–1465, doi:10.1126/science.abb0074 (2020). [PubMed: 32327602]
15. Lessard J. et al.An essential switch in subunit composition of a chromatin remodeling complex during neural development. Neuron55, 201–215, doi:10.1016/j.neuron.2007.06.019 (2007). [PubMed: 17640523]
16. Staahl BTet al.Kinetic analysis of npBAF to nBAF switching reveals exchange of SS18 with CREST and integration with neural developmental pathways. J Neurosci33, 1034810361, doi:10.1523/JNEUROSCI.1258-13.2013 (2013).

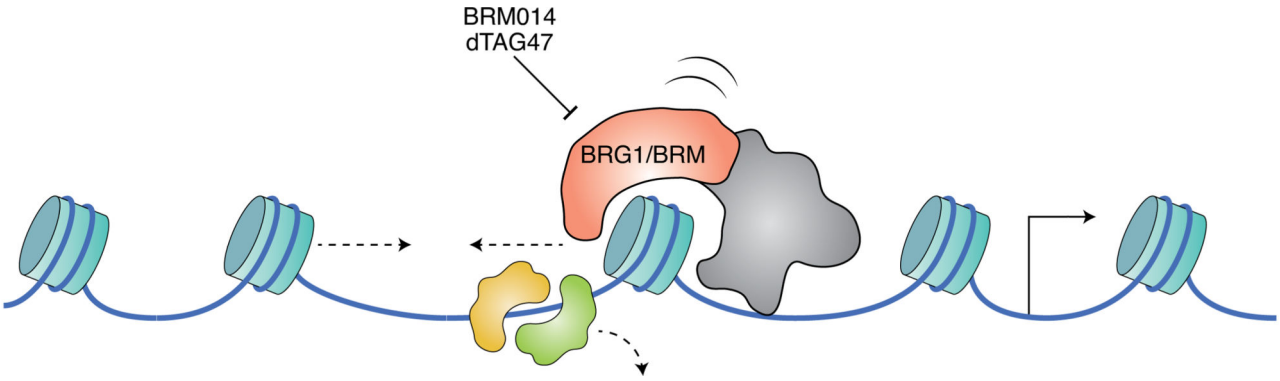

Figure 1 –. SWI/SNF activity is needed to maintain accessible chromatin.

Acute disruption of the BRG1/BRM ATPases of the SWI/SNF complex results in loss of chromatin accessibility and transcription factor binding at enhancers within minutes, indicating that SWI/SNF-dependent chromatin remodeling is continuously required to maintain open chromatin for transcription. Yellow/green: OCT4, SOX2 transcription factors; Pink: BRG1/BRM subunit of the SWI/SNF complex; Gray: Associated SWI/SNF subunits; Blue: nucleosomes.