Dynamics of BAF–Polycomb complex opposition on heterochromatin in normal and oncogenic states

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The opposition between Polycomb repressive complexes (PRCs) and BAF (mSWI/SNF) complexes has a critical role in both development and disease. Mutations in the genes encoding BAF subunits contribute to more than 20% of human malignancies, yet the underlying mechanisms remain unclear, owing largely to a lack of assays to assess BAF function in living cells. To address this, we have developed a widely applicable recruitment assay system through which we find that BAF opposes PRC by rapid, ATP-dependent eviction, leading to the formation of accessible chromatin. The reversal of this process results in reassembly of facultative heterochromatin. Surprisingly, BAF-mediated PRC eviction occurs in the absence of RNA polymerase II (Pol II) occupancy, transcription, and replication. Further, we find that tumor-suppressor and oncogenic mutant BAF complexes have different effects on PRC eviction. The results of these studies define a mechanistic sequence underlying the resolution and formation of facultative heterochromatin, and they demonstrate that BAF opposes PRC on a minute-by-minute basis to provide epigenetic plasticity.

The portion of the genome that may be subject to regulatory mechanisms appears to reflect a balance between chromatin processes that favor chromatin accessibility and those that oppose it. This balance was first recognized in Drosophila melanogaster, in which the Trithorax group of genes was shown to favor activation of developmental genes, while Polycomb genes were found to oppose this activation1. The Trithorax genes encode members of the BAP (Brahma-associated protein) ATP-dependent chromatin-remodeling complex and enzymes that produce the activating histone modification trimethylation of histone H3 at lysine 4 (H3K4me3)2–4. Genetically, Trithorax proteins act in opposition to Polycomb genes, which encode the subunits of the Polycomb repressive complex 1 (PRC1) and 2 (PRC2). PRC1 and PRC2 direct histone H2A ubiquitination (H2AK119ub1) and trimethylation of histone H3 at lysine 27 (H3K27me3), respectively, favoring histone H3 at lysine 4 (H3K4me3)2–4. Genetically, Trithorax proteins act in opposition to Polycomb genes, which encode the subunits of the Polycomb repressive complex 1 (PRC1) and 2 (PRC2). PRC1 and PRC2 direct histone H2A ubiquitination (H2AK119ub1) and trimethylation of histone H3 at lysine 27 (H3K27me3), respectively, favoring inaccessible chromatin5. The presence of PRC1 and PRC2 is a mark of ‘facultative heterochromatin’, which is distinguished from constitutive heterochromatin at centromeres and other regions of the genome.

Genomic studies have shown that the genes involved in creating the opposition between processes favoring and opposing chromatin accessibility are frequently mutated in human cancer. Subunits of the mammalian SWI/SNF or BAF (Brg/Brm-associated factor) complexes are mutated in more than 20% of all human cancers6,7 and a large number of human neurologic diseases8–11. These complexes promote accessibility, at least in part, by opposing the actions of Polycomb complexes12,13. The MLL genes encode catalytic subunits of the COMPASS complex that place the activation-associated H3K4me3 modification14. These genes are mutated in a large number of somatic cancers4. The catalytic PRC2 subunit EZH2 is mutated or silenced in a number of leukemias and lymphomas15–17.

In mammals, BAF complexes are 15-subunit assemblies that comprise different combinations of proteins, which are encoded by 29 genes. These highly polymorphic complexes can be exquisitely cell type specific, such as the nBAF complex found only in postmitotic neurons18,19. The BAF subunit mutations found in human cancer have a striking pattern of tissue specificity. For example, nearly 100% of cases of human synovial sarcoma result from the SS18-SSX (t(X;18) translocation (where SSX can be SSX1, SSX2, or SSX4); however, the SS18 BAF subunit is rarely mutated in other cancers. Malignant rhabdoid tumors (MRTs) uniformly result from deletions or loss-of-function mutations in SMARCB1 (also known as BAF47, INI1, or NF5); but the SMARCB1 subunit is less frequently involved in other human cancers20. The present data indicate that the mechanisms of oncogenesis appear to relate to the ability of BAF complexes to oppose Polycomb-mediated repression. In human MRTs, loss of SMARCB1 leads to Polycomb-mediated repression of genes that suppress proliferation, such as CDKN2A21, while re-expression of SMARCB1 leads to removal of Polycomb from chromatin and loss of DNA methylation by unknown mechanism(s)22. Long time courses of re-expression in these earlier experiments were informative but did not allow for direct mechanistic analysis of the loss of Polycomb from chromatin, as loss could have been caused by differentiation, replication, or other cell biological actions. Nevertheless, the results of correlative studies suggest...
that BAF might evict Polycomb at the CDKN2A locus. Conversely, in synovial sarcoma, the SS18-SSX oncogenic fusion protein, which is the product of the oncogenic allele, dominantly assembles into BAF complexes, targeting them to silenced Polycomb target genes where the altered BAF complex appears to remove Polycomb repression. However, it is not known whether the BAF–Polycomb balance is achieved directly or indirectly, nor is there any knowledge of a causal sequence of biochemical events that provide this critical balance.

The mechanism underlying BAF–Polycomb opposition has been difficult to study. This is because present in vitro approaches using nucleosomal templates are unable to mimic the effects of tissue-specific histone modifications, long-range interactions, topological features, and post-translational modifications of the proteins involved. To elucidate the mechanism of BAF–Polycomb complex opposition, we developed a method to rapidly and reversibly recruit a chromatin regulator of interest to one allele of an endogenous gene and then measure and model the sequence of biochemical events that occur at this locus. We find that BAF complex recruitment evicts both PRC1 and PRC2 within 5 min and the development of chromatin accessibility follows. The order of deletion and reappearance predicts that PRC1 activity precedes PRC2 activity. This study shows that, in contrast to some expectations, BAF complexes oppose both PRC1 and PRC2 on a minute-by-minute basis without need for replication, Pol II occupancy, or transcription.

RESULTS
Development of an assay system to study the mechanism of BAF–Polycomb opposition
To study the opposition between BAF and Polycomb complexes at repressed facultative heterochromatin, we modified the endogenous Pou5f1 (also known as Oct4) locus, which in mouse embryonic fibroblasts (MEFs) is repressed by both Polycomb complexes24,25 and trimethylation of histone H3 at lysine 9 (H3K9me3)26 and lacks BAF complex occupancy. By contrast, in pluripotent stem cells, the locus lacks Polycomb binding and instead has robust SMARCA4 (also known as BRG1) binding over the proximal enhancer, which

Figure 1 Design and development of a rapidly inducible system to recruit BAF complexes to heterochromatin in living cells. (a) Schematic of recruitment of FRB-tagged BAF complexes by rapamycin in MEFs to the Pou5f1 locus. Rapamycin dimerizes with FRB and FKBP. Primer distances correspond to distances from the Pou5f1 transcription start site (TSS). (b) Mouse embryos with the recruitment system and a CiA-modified Pou5f1 allele (GFP) exhibit normal embryonic development and similar Pou5f1 expression from the inserted allele and the wild-type allele in blastocysts. OCT4 expression was analyzed at the protein level. Scale bar, 10 μm. (c) ChiP–qPCR and ChiP–seq for repressive and activating marks indicate large-scale repression of the Pou5f1 locus. Data are shown as means ± s.d. for n = 10 experiments. (d) Total nuclear input and SMARCA4 immunoprecipitation (IP) showing that FRB-V5-SS18 properly assembles into BAF complexes. Endo, endogenous. (e) BAF complex recruitment (ChiP–qPCR fold enrichment relative to no rapamycin treatment at the ZFHD1 site at −443 bp) can be achieved using several different FRB-tagged BAF subunits. BAF complex recruitment reaches saturation by 1.5 h. (f) SMARCA4 and SMARCC1 ChiP–qPCR demonstrate that FRB-V5-SS18 recruits the complete BAF complex to the ZFHD1 recruitment site (−443 bp). (g) Landscape plot of V5 ChiP–qPCR data demonstrating BAF complex regional occupancy over a 60-min time course. The spread of the BAF domain is 920 ± 305 bp with respect to the ZFHD1 recruitment site (95% CI). Data are shown as means ± s.d. for n = 3 experiments in e–g.
is essential for Pou5f1 regulation\(^{12,27-29}\) (Supplementary Fig. 1a). To analyze the resolution of heterochromatin by BAF, we developed the CiAO (chromatin indicator and assay at Oct4) mouse by modifying one Pou5f1 allele to have two different arrays of transcription factor binding sites upstream of the transcription initiation site\(^{30}\) (Fig. 1a and Supplementary Fig. 1b). In addition, the GFP coding sequence was inserted into the Pou5f1 allele, allowing the visualization of Pou5f1-expressing cells, but inactivating one allele. The allele containing the insertions is regulated similarly, both qualitatively and quantitatively, to the unmodified allele. In addition, the histone modification landscape of the modified allele is indistinguishable from that of the unmodified allele (Fig. 1b and Supplementary Fig. 1c). These observations indicate that both local and long-range topological features are not disturbed on the modified allele and that the insertions do not modify the pattern of histone modifications.

The Pou5f1 allele containing the insertions is active in both pluripotent and germ cells derived from the CiAO mouse, but it is intensely repressed by Polycomb-group marks such as H3K27me3 and H3K9me3 in fibroblasts (Fig. 1c). The Pou5f1 locus undergoes repression upon embryonic stem cell (ESC) differentiation\(^{30}\), and in fibroblasts the gene can only be activated after prolonged exposure to the core pluripotency factors\(^{31}\).

This system provides a broadly applicable model for developmental chromatin regulation, allowing the temporally precise addition of one or two specific factors within a context of normal chromatin. While signaling pathways such as the LIF–STAT3 cascade also induce chromatin changes, LIF responses are too diverse, involving many chromatin regulators, and too asynchronous to allow mechanistic interpretation\(^{12,32}\). We used a chemical inducer of proximity (CIP), rapamycin, which induces proximity of proteins at the modified Pou5f1 allele by virtue of its ability to bind one protein tag (FRB) on one side and another tag (FKBP) on the other side of the rapamycin small molecule (Fig. 1a). Because rapamycin binding is limited by diffusion and the off-rate is on the order of seconds, this approach does not produce a rigid topology, but rather a cloud of complexes\(^{33,34}\). This is in contrast to direct fusions, which produce rigid conformations that can sterically restrict the activity of the recruited proteins. Thus, the recruited BAF complex is free to assume its normal mode of binding to the Pou5f1 locus. To induce proximity of the BAF complex, we chose to fuse the SS18 subunit to FRB because SS18

Figure 2  BAF complexes displace PRC repression upon recruitment. (a) Schematic for rapamycin-induced recruitment of FRB-V5-SS18 BAF complexes. (b,c) BAF complex recruitment results in EZH2 displacement within 10 min followed by H3K27me3 removal within 20 min (b) and RING1B displacement within 5 min followed by H2AK119ub1 removal within 7.5 min (c) (at the ZFHD1 site at −443 bp). (d) Left, total histone H3 occupancy and non-PRC histone marks (H3K9me3, H2A.Z) are unaffected by BAF complex recruitment. Right, comparison of histone H3 levels to PRC marks shows removal of PRC1 repression followed by removal of PRC2 repression, with histone H3 levels unchanged. (e) ATAC-qPCR at the ZFHD1 recruitment site (−443 bp) shows an increase in DNA accessibility upon BAF complex recruitment. (f) The HELLS (LSH) chromatin remodeler shows rapid recruitment via the rapamycin recruitment system (left); however, PRC1 and PRC1-placed repressive marks are not displaced, nor are H3K9me3 or total histone H3 (right). All ChIP–qPCR measurements are at the ZFHD1 site at −443 bp. Data are shown as means ± s.d. for \(n = 3\) experiments, except in c where \(n = 2\) experiments. All \(P\) values were determined using a two-tailed \(t\) test in PRISM 7.
Rapid removal of BAF complexes by competitive inhibition of rapamycin triggers reformation of repressed heterochromatin. (a) Schematic for FK1012-driven washout of rapamycin-tethered BAF complexes. (b) Comparison of BAF occupancy upon FK1012-driven washout to that observed with no washout (medium exchange). (c,d) FK1012 washout triggers show reformation of heterochromatin, with PRC2 (c) and PRC1 (d) repression beginning to reform within 2–5 min of rapamycin addition (Fig. 1f). We found that recruitment of BAF led to the removal of both the PRC2 (EZH2) and the H3K27me3 mark within minutes (Supplementary Fig. 1f). On the basis of published studies, we calculated the fractional occupancy to be about 20% and the dwell time to be <83 s, indicating that the recruited complexes are maintained at this location in part by direct interactions with facultative heterochromatin. Thus, using this CIP system, BAF complexes can be recruited within minutes, at normal levels and with normal temporal dynamics.

Recruitment of BAF complexes results in rapid eviction of PRC complexes

Mutations of the Drosophila BAP (dSWI/SNF) ATPase brm entirely suppresses the effects of PRC1 mutations on body plan morphogenesis, attesting to the remarkable functional dedication of BAF and Polycomb13,37,38. Notably, disruption of the BAF–Polycomb opposition has become increasingly recognized as an oncogenic mechanism in several human cancers21,23,39. Therefore, we first measured the effect of BAF recruitment on PRC eviction (Fig. 2a). We found that recruitment of BAF led to the removal of both the PRC2 complex (EZH2) and the H3K27me3 mark within minutes (Fig. 2b and Supplementary Fig. 2a). We also tested the alternative possibility that BAF recruitment removes PRC2 with subsequent loss of H3K27me3 by comparing the time courses of PRC2 and H3K27me3 removal after recruitment of the BAF complex. Unexpectedly, we found a full 10-min lag between the removal of PRC2 (EZH2) and the initial reduction of H3K27me3 levels (t(lag): 9.22 < t < 11.41 min) (Fig. 2b).

Remarkably, addition of 3 nM rapamycin recruited the entire 2-MDa BAF complex to the Pou5f1 locus with a lag time of only 2 min (2.2 < t < 4.8 min, 95% confidence interval (CI)) at levels similar to BAF peaks over the genome of ESCs (Supplementary Fig. 1g). To be certain that the complexes were fully assembled, we performed ChIP experiments using antibodies to V5 (to capture the complexes bearing FRB-V5-SS18), as well as SMARCA4 and SMARCC1 (also known as BAF155). We confirmed that each of these components was effectively recruited within 2–5 min of rapamycin addition (Fig. 1f and Supplementary Fig. 1h). Notably, the levels and extent of BAF binding when the rapamycin concentration was 3 nM were similar to those for BAF peaks over the genome27. BAF complexes occupied a region of approximately 1,200 bp, consistent with binding of a single 2-MDa complex6 (Fig. 1g). The time courses of PRC2 and H3K27me3 removal after recruitment of the BAF complex were determined that, within 24 h, BAF complex recruitment was induced with no washout (medium exchange). (e) ATAC–qPCR at the recruitment site shows that accessibility is lost upon FK1012 washout and reformation of heterochromatin. All ChIP–qPCR measurements were at the ZFHD1 site at −443 bp. Data in b–e are shown as means ± s.d. for n = 3 experiments.
This lag-time is unlikely to reflect differences in antibody detection, as the histone modification is more abundant than the enzyme.

PRC2 works in synergy with PRC1 to repress genes, and both Polycomb complexes and their associated histone marks are present at the repressed Pou5f1 locus in fibroblasts. In flies, mutations in PRC1 (pc1 or CBX6 subunit) are nearly completely repressed by mutations in the brm ATPase, demonstrating their opposition with one another.

Remarkably, PRC1 disappeared from the repressed Pou5f1 locus even more quickly than PRC2, as assayed by ChIP using an antibody that recognizes RING1B (Fig. 2c). Eviction of PRC1 was paralleled by dissolution of the H2AK119ub1 mark (histone H2 ubiquitinated at lysine 119) at this locus (Fig. 2c). Decreased occupancy of the H2AK119ub1 mark preceded the decreased occupancy of the H3K27me3 mark (Fig. 2d).

These observations raised the question of whether BAF-recruitment-mediated PRC displacement results from an increased rate of nucleosome or histone exchange. Previous studies have shown that BAF complexes can exchange nucleosomes in vitro, but this possibility has not been tested in vivo.

We found that, within the first hour, there was no detectable change in the levels of H3K9me3 or histone H2A.Z, suggesting that the removal of H3K27me3 resulting from BAF complex recruitment does not reflect a non-specific enhancement of nucleosomal turnover (Fig. 2d and Supplementary Fig. 2b). This is consistent with our observation that deletion of BAF subunits, SMARCA4 and ACTL6A (also known as BAF53A) does not result in detectable genome-wide changes in nucleosome occupancy or placement as assayed by MNase–seq or ATAC–seq (E.L.M., D.C. Hargreaves, C.K., C. Chang, and J.P.C., unpublished data).

Because we unexpectedly could not detect histone H3 depletion after BAF complex recruitment, we developed another mouse model system by double knock-in of the GAL4 and ZnDB DNA-binding sites and GFP reporter at the Ascl1 locus, which we call the CiaA mouse (chromatin indicator and assay at Ascl1) mouse. Here we used the Ascl1 locus, which encodes the neuronal pioneer factor ASCL1. Ascl1 has both H3K27me3 and H3K4me3 marks over its regulatory regions and has a CpG island, which is often seen at PRC-marked sites.

The Ascl1 promoter is occupied by RNA Pol II and is accessible (according to the Encyclopedia of DNA Elements (ENCODE) data). Hence, the histone marks of the Ascl1 locus in ES cells are quite different from those at Pou5f1 in fibroblasts. This difference allowed us to test the robustness of our findings to different loci over the genome.

Addition of rapamycin resulted in robust recruitment of BAF to the Ascl1 locus (Supplementary Fig. 2c). Similarly to Pou5f1 in MEFs, we found that BAF recruitment led to eviction of PRC1 and PRC2 within 2 min. In contrast to the highly repressed Pou5f1 locus in MEFs, at the Ascl1 locus in ESCs, we were able to detect histone H3 turnover using CATCH-IT analysis and also to observe depletion of histone H3 by ChIP analysis (Supplementary Fig. 2c).

Thus, it appears that Polycob eviction occurs without detectable histone H3 or H3K9me3 depletion at the facultative heterochromatin of the Pou5f1 locus in MEFs, but histone H3 exchange is clearly detectable in ESCs at the accessible Ascl1 locus upon BAF recruitment. However, the time course of PRC1 eviction and the time course of histone H3 exchange at the Ascl1 locus were nearly identical, preventing us from assigning causality to either process at this locus.

We predicted that, if Polycob contributed substantially to the repression of the Pou5f1 locus, we would find enhanced accessibility over the recruitment sites corresponding to either removal of the H3K27me3 mark or Polycob complexes. We assayed chromatin accessibility using a modified ATAC–seq assay that measures the ability of the Tn5 transposase to invade open, but not closed, chromatin (Supplementary Fig. 2c).

Figure 4 BAF complex-mediated eviction of Polycomb is ATP dependent. (a) SMARCA4-V5-FRB system for rapidly recruiting BAF complexes containing wild-type SMARCA4 or ATPase-dead SMARCA4 (Lys785Arg mutant). (b) BAF complex recruitment to the ZFHD1 recruitment site with wild-type (WT) and ATPase-dead SMARCA4 is comparable. (c,d) Eviction of PRC proteins is dependent on ATPase function, with EZH2 (c) and RING1B (d) eviction reduced with ATPase-dead SMARCA4 as compared to wild-type SMARCA4. All ChIP–qPCR measurements are at the ZFHD1 site at −443 bp. Data are shown in b–d as means ± s.d. for n = 3 experiments: **P < 0.01.
the underlying mechanisms, we studied the reassembly of Polycomb-repressed heterochromatin. This was achieved using FK1012 (ref. 34), a dimeric competitive inhibitor of rapamycin, which binds only to the FKBP side. FK1012 competed with rapamycin, rapidly removing it from the complex (Fig. 3a and Supplementary Fig. 3a). In comparing the kinetics of rapamycin washout (via medium change) to the kinetics following addition of FK1012, we determined that addition of FK1012 resulted in more rapid, robust decreases in BAF complex tethering to the DNA. To determine whether inaccessible heterochromatin could be reformed, we found that addition of FK1012 led to both the removal of BAF complexes within 0.5 < t < 30 min (Fig. 3b), as assessed by anti-V5 ChIP, and the reappearance of PRC2 (EZH2) and H3K27me3 by 0.5 < t < 2.5 h (Fig. 3c). We found that PRC2 (EZH2) and PRC1 (RING1B) complexes began to reappear within ~2 h of the addition of FK1012 and that this was paralleled by the reappearance of H3K27me3 and H2Aub1 (Fig. 3c). The open, DNA-accessible state produced by BAF complex dissociation was not stable, as suggested by in vitro studies on nucleosomal templates47; rather, inaccessible chromatin began to reform within 2.5–5 h of removal of the BAF complex (Fig. 3c and Supplementary Fig. 3b–e). These washout experiments mimic the developmental transition that occurs over many genes that are active in early development and later become repressed by Polycomb and facultative heterochromatin. Thus, our system allows one to make kinetic determinations in living cells of both dissolution and establishment of facultative heterochromatin.

Eviction of PRC and associated histone marks is dependent on the ATPase activity of SMARCA4

The ATPase activity of BAF complexes is provided by the SMARCA4 or SMARCA2 (also known as BRM) subunit, which is necessary for the function of BAF complexes in a variety of assays, and the ATPase domains are frequently mutated in cancer and neurologic diseases39,48. Hence, we asked whether the ATPase activity of SMARCA4 was necessary for PRC1 and PRC2 eviction. To examine this, we directly recruited SMARCA4 by fusing the FRB tag to the C terminus of the protein. Recruitment of the BAF complex by this strategy was not as robust as it was with the fusion to SS18. However, we did find that the SMARCA4 fusion resulted in about a 4- to 8-fold increase in occupancy of SMARCC1 (BAF155) at the recruitment site as compared to the 40- to 60-fold increase seen with the SS18 fusion. To test the role of the ATPase activity of SMARCA4, we used a mutant (Lys785Arg) with reduced ATPase activity49 that we originally reported by our group and is also found in a number of cancers and neurologic diseases48. Recruitment of this mutant SMARCA4 protein to the Pou5f1 locus in MEFs (Fig. 4a,b) led to less PRC1 and PRC2 eviction than found with wild-type SMARCA4 (Fig. 4c,d). Thus, the ATPase activity of SMARCA4 is required for PRC eviction. This result, along with the HELLS recruitment studies (Fig. 2f), rules out the possibility that non-specific steric occlusion contributes to PRC eviction. The experiments above indicate that BAF complexes are capable of driving a transition from inaccessible higher-order chromatin structure toward accessibility, and that this transition is due to the direct eviction of both PRC1 and PRC2.

Recruitment of cancer-specific BAF complexes to repressed heterochromatin

BAF complexes can behave as either oncogenes or tumor suppressors. Unfortunately, it has not been possible to directly assay the effects of these tumor-suppressor or oncogenic mutations using in vitro assays. Hence, we asked whether we would be able to discern the mechanism of these oncogenic mutations using the CiAO assay. To this end, we recruited BAF complexes with highly specific driver mutations of complex subunits, which define specific cancer
subtypes with Polycomb-repressed chromatin. To study the consequences of recruitment of BAF complexes lacking the SMARCB1 tumor-suppressor subunit, the hallmark feature of pediatric MRTs, we performed short hairpin RNA (shRNA)-mediated knockdown of Smarcb1 we performed short hairpin RNA (shRNA)-mediated knockdown of Smarcb1 (knockdown efficiency >80%). We then recruited BAF complexes, using SMARCE1 as the FRB-V5-tagged subunit in this case because Smarcb1 knockdown results in slightly reduced SS18 binding to BAF complexes (Figs. 1c and 5a, and Supplementary Fig. 1d). Complexes tagged with FRB-V5-SMARCE1, both wild type and depleted of SMARCB1, displayed comparable recruitment levels to the Pou5f1 locus (Fig. 5b,c). Intriguingly, however, SMARCB1-depleted complexes exhibited significantly decreased ability to displace EZH2 (PRC2 complexes), RING1B (PRC1 complexes), and the H3K27me3 mark at the zinc-finger binding domain when compared to wild-type complexes (Fig. 5d-f). This suggests that SMARCB1 loss in tumors leads to an inability to oppose Polycomb, mechanistically explaining the tumor-suppressive functions previously observed at CDKN2A and other loci11 and supporting the therapeutic use of PRC inhibitors.

BAF complexes can also be oncogenes that both initiate and drive cancer, as is the case with the SS18-SSX translocation that is found in nearly 100% of synovial sarcomas and in nearly 100% of the cells within the tumor. Hence, we sought to determine whether BAF complexes with the SS18-SSX fusion protein could oppose Polycomb. To perform these studies, we developed FRB-V5-SS18-SSX fusions for which we directly compared the results with our measurements using FRB-V5-SS18 (wild type) (Fig. 6a). Using anti-SMARCA4 immunoprecipitation, we demonstrated that these complexes bear the expected features of BAF complexes containing the SS18-SSX fusion as demonstrated previously23, including reduced protein assembly of both SMARCB1 and wild-type SS18 (Fig. 6b). Notably, SS18-SSX BAF complexes displayed a dramatically extended domain of BAF occupancy, spreading 2,620 ± 456 bp (95% CI) into the Pou5f1 gene body, and as compared to complexes with wild-type SS18 (920 ± 305 bp (95% CI)), likely reflecting gained multimerization or processivity of the complexes (Fig. 6c). While BAF complex recruitment at the zinc-finger recruitment site (+0 bp) was comparable for wild-type SS18 and the SS18-SSX fusion over a 60-min time course (Supplementary Fig. 4a), BAF complex occupancy at downstream sites >1,000 bp into the gene body was achieved only by SS18-SSX oncogenic BAF complexes (Fig. 6c and Supplementary Fig. 4c). Notably, SS18-SSX oncogenic BAF complexes robustly displaced both PRC2 and PRC1 complexes (Fig. 6d,e and Supplementary Fig. 4b,c,e,f), as well as the H3K27me3 repressive mark (Fig. 6f and Supplementary Fig. 4d,h), at sites located at +1,034 bp and +2,287 bp with respect to the ZFHD1 recruitment site, while wild-type SS18 complexes were unable to achieve these effects outside of the 1,000-bp region flanking the ZFHD1 recruitment site. These results explain the robust removal of PRC2 and H3K27me3 over the entire SOX2 gene observed in synovial sarcoma.

DISCUSSION

Our studies indicate that the mechanism by which BAF complexes oppose Polycomb complexes is at least in part achieved through rapid eviction of PRC1 and PRC2 (Fig. 7). The ATPase activity of SMARCA4 is required for eviction, suggesting that the process is specific and pointing toward possible mechanisms by which ATPasedefault mutants act in human cancers. The fact that eviction occurs within 2–5 min of BAF recruitment indicates that neither cell replication nor transcription is necessary for Polycomb complex removal. These results illustrate the power of the CiA20 system, which enables precise temporal control over the kinetics of BAF–Polycomb opposition. Because we could not detect the expected enhanced rates of nucleosome turnover for either histone H3 or H3K9me3, we speculate...
that loss of H3K27me3 reflects the natural rates of decay due to histone demethylases and basal rates of nucleosome removal\textsuperscript{44}. Indeed, BAF has been reported to bind to H3K27 demethylases\textsuperscript{50}, suggesting that it might recruit these enzymes to its sites of action. Chromatin accessibility rapidly follows the loss of H3K27me3 and H2Aub1, as expected from previous studies. In our G10 system, we essentially modify the chromatin landscape of the Pou5f1 gene in MEFs to be more like that in ESCs, in which the gene is active and covered by a large domain of BAF. By removing the CIP through competition with PK1012, we revert the locus to one with inaccessible chromatin consistent with continuous opposition between BAF and Polycomb complexes, rather than a stable expression state based on nucleosome structure.

The mechanism of action that we describe in which BAF prepares a Polycomb-repressed locus for binding of transcription factors (Fig. 7) provides an explanation for the apparent instructive functions of specific BAF complexes. For example, switching the subunit composition to that of the neural-specific nBAF complex in human fibroblasts converts cells to a basal neuronal state that can be biased with specific transcription factors to produce types of neurons that have never been derived in culture from either ESCs or fibroblasts\textsuperscript{31–33}. Instructive roles have also been reported in induced pluripotent stem cell (iPSC) conversion\textsuperscript{34}, the heart field\textsuperscript{35}, the wiring of the Drosophila olfactory system\textsuperscript{56}, and induction of specific types of neurons in Caenorhabditis elegans\textsuperscript{57}. Our model (Fig. 7) does not reduce the need for sequence-specific or lineage-specific transcription factors in these biological processes, but rather suggests that BAF and its tissue-specific assemblies act to open the range of possible binding sites for such factors and may possibly also aid in the positioning of nucleosomes to allow transcription factor binding.

Our studies indicate that loss of the SMARCB1 tumor-suppressor subunit, observed in MRTs, leads to substantially diminished eviction (Fig. 7f). This mechanism predicts the observations in malignant cells suggesting that loci that repress proliferation, such as CDKN2A, become intensely repressed by a domain of H3K27me3 that builds over this gene, leading to a failure to halt cell division\textsuperscript{21}.

The SS18-SSX fusion protein, which both initiates and drives synovial sarcoma, is an example of an instructive oncogenic function of an altered BAF complex\textsuperscript{23}. Addition of only 78 amino acids of SSX onto the C terminus of the SS18 subunit leads to preferential assembly of the fusion protein into an oncogenic BAF complex that then targets the inactive SOX2 locus. The resulting complex removes Polycomb and activates expression of the SOX2 gene, thereby driving proliferation. This sequence of events largely precludes a mechanism in which a transcription factor recruits BAF because the SOX2 locus is inactive in the cell type that gives rise to the malignancy and the oncogenic BAF complex can activate the SOX2 gene in fibroblasts, in which the SOX2 locus is inactive and likely not occupied by transcription factors (Fig. 7g). Our direct recruitment studies indicate that the role of the SS18-SSX fusion is to produce a complex that propagates along the chromosome to occupy a larger region than is normally occupied by BAF over the SOX2 gene in cells in which this gene is inactive. We find this larger region of occupancy in both BAF ChIP-seq studies in the malignant synovial sarcoma cells that
bear the translocation23 and also when we recruit the complex to the silent Pou5f1 locus in MEFs. Propagation of the complex leads to a larger domain of Polycrom removal and, hence, a greater chance that a transcription factor present in fibroblasts will bind to the now-accessible chromatin prepared by the oncogenic BAF complex. This scenario illustrates how these complexes can assume an instructive function (in this case, that function is uncontrolled proliferation) by allowing transcription factors present in fibroblasts to activate a gene normally only active in pluripotent cells and neural progenitors.

In the same way, the nBAF complex might prepare neural-specific genes for activation during reprogramming of fibroblasts to neurons51,52. Thus, our studies provide an explanation for both the tumor-suppressor (SMARCB1 deletion in MRT) and oncogenic (SS18-SSX fusion in synovial sarcoma) functions of BAF complexes.

Recent exome sequencing studies have highlighted striking frequencies of mutations in both BAF and Polycrom subunits in human cancers39,58. Where studied, mutations in subunits of BAF complexes identify extensive roles in human malignancy. 

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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We would like to dedicate this paper to Joe Calarco, who died tragically during the course of this work. His energy, enthusiasm, and insight will continue to guide our work and his warmth brightens our memories.

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**COMPETING FINANCIAL INTERESTS**

The authors declare competing financial interests: details are available in the online version of the paper.

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1. Kennison, J.A. & Tamkun, J.W. Trans. regulation of homeotic genes in Drosophila. New Biol. 4, 91–96 (1992).
2. Simon, J.A. & Kingston, R.E. Occupying chromatin: Polycrom mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. Mol. Cell 49, 808–824 (2013).
3. Morgan, M.A. & Shilatifard, A. Chromatin signatures of cancer. Genes Dev. 29, 238–248 (2015).
4. Piunti, A. & Shilatifard, A. Epigenetic balance of gene expression by Polycrom and COMPASS families. Science 352, 109780 (2016).
5. Margueron, R. & Reinberg, D. The Polycrom complex PR2C and its mark in life. Nature 469, 343–349 (2011).
6. Kadoch, C. et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. Nat. Genet. 45, 592–601 (2013).
7. Shain, A.H. & Pollack, J.R. The spectrum of SWI/SNF mutations, ubiquitous in human cancers. PLoS One 8, e55119 (2013).
8. Kosho, T. & Okamoto, N. Germline-phenotype correlation of Coffin–Siris syndrome caused by mutations in SMARCB1, SMARCA4, SMARCE1, and ATRIDIA. Am. J. Med. Genet. C, Semin. Med. Genet. 166C, 262–275 (2014).
9. Santen, G.W. et al. Mutations in SWI/SNF chromatin remodeling complex gene ARID1B cause Coffin–Siris syndrome. Nat. Genet. 44, 379–380 (2012).
10. Tsurskii, Y. et al. Mutations affecting components of the SWI/SNF complex cause Coffin–Siris syndrome. Nat. Genet. 44, 376–378 (2012).
11. Deciphering Developmental Disorders Study. Large-scale discovery of novel genetic causes of developmental disorders. Nature 519, 223–228 (2014).
12. Lo, L. et al. esBAF facilitates pluripotency by conditioning the genome for LIF/STAT3 signalling and by regulating Polycrom function. Nat. Cell Biol. 13, 903–913 (2011).
13. Tamkun, J.W. et al. Brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF/SWI. Cell 68, 561–572 (1992).
14. Tkachuk, D.C., Kohler, S. & Cleary, M.L. Involvement of a homolog of Drosophila Trithorax by 11q23 chromosomal translocations in acute leukemias. Cell 71, 691–700 (1992).
15. Kim, K.H. & Roberts, C.W. Targeting EZH2 in cancer. Nat. Med. 22, 128–134 (2016).
16. Varambally, S. et al. The Polycrom group protein EZH2 is involved in progression of prostate cancer. Nature 432, 624–629 (2004).
17. Lee, W. et al. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. Nat. Genet. 46, 1227–1232 (2014).
18. Wu, J.J., Lessard, J. & Crabtree, G.R. Understanding the words of chromatin regulation. Cell 136, 200–206 (2009).
19. Wu, J.J. et al. Regulation of dendritic development by neuron-specific chromatin remodeling complexes. Neuron 56, 94–108 (2007).
20. Versteege, I. et al. Truncating mutations of NSKSN51IN1 in aggressive paediatric cancer. Nature 394, 203–206 (1998).
21. Wilson, B.G. et al. Epigenetic antagonism between Polycrom and SWI/SNF complexes during oncogenic transformation. Cancer Cell 18, 316–328 (2010).
22. Kia, S.K., Gorski, M.M., Giannakopoulou, S. & Verrijzer, C.P. SWI/SNF mediates Polycrom eviction and epigenetic reprogramming of the INK4d-ARF-INK4a locus. Mol. Cell Biol. 28, 3457–3464 (2008).
23. Kadoch, C. & Crabtree, G.R. Reversible disruption of mSWI/SNF (BAF) complexes by the SS18-SSX oncogenic fusion in synovial sarcoma. Cell 153, 71–85 (2013).
24. Li, G. et al. Jarid2 and PRC2, partners in regulating gene expression. Genes Dev. 24, 388–380 (2010).
25. van der Stoop, P. et al. Ubiquitin E3 ligase Ring1b/Rnf2 of Polycrom repressive complex 1 contributes to stable maintenance of mouse embryonic stem cells. PLoS One 3, e2325 (2008).
26. Feldman, N. et al. G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. Nat. Cell Biol. 8, 181–194 (2006).
27. Ho, L. et al. An embryonic stem cell chromatin remodeling complex, esBAF, is an essential component of the core pluripotency transcriptional network. Proc. Natl. Acad. Sci. USA 106, 5187–5191 (2009).
28. Ho, L. et al. An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. Proc. Natl. Acad. Sci. USA 106, 5181–5186 (2009).
29. Young, R.A. Control of the embryonic stem cell state. Cell 144, 940–954 (2011).
30. Nakahata, N.A. et al. Dynamics and memory of heterochromatin in living cells. Cell 149, 1447–1460 (2012).
31. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676 (2006).
32. Crabtree, G.R. Olson, E.N. NFAT signaling: structuring the social lives of cells. Cell 109 (Suppl.), S67–S79 (2002).
33. Crabtree, G.R. & Schreiber, S.L. Three-part inventions: intracellular signaling and induced proximity. Trends Biochem. Sci. 21, 418–422 (1996).
34. Spencer, D.M., Wandiess, T.J., Schreiber, S.L. & Crabtree, G.R. Controlling signal transduction with synthetic ligands. Science 262, 1019–1024 (1993).
35. de Bruijn, D.R. et al. Targeted disruption of the synovial sarcoma-associated SS18 gene causes early embryonic lethality and affects PPARBP expression. Hum. Mol. Genet. 15, 2936–2944 (2006).
36. Banaszynski, L.A., Liu, C.W. & Wandiess, T.J. Characterization of the FKBP12rapamycin-FRB ternary complex. J. Am. Chem. Soc. 127, 4715–4721 (2005).
37. Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B. & Cavalli, G. Genome regulation by Polycrom and Trithorax proteins. Cell 128, 735–745 (2007).

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38. Blackledge, N.P. et al. Variant PRC1 complex–dependent H2A ubiquitylation drives PRC2 recruitment and Polycomb domain formation. Cell 157, 1445–1459 (2014).
39. Kadoch, C. & Crabtree, G.R. Mammalian SWI/SNF chromatin remodeling complexes and cancer: mechanistic insights gained from human genomics. Sci. Adv. 1, e1500447 (2015).
40. Yen, K., Vinayachandran, V., Batta, K., Koerber, R.T. & Pugh, B.F. Genome-wide nucleosome specificity and directionality of chromatin remodelers. Cell 149, 1461–1473 (2012).
41. Lorch, Y., Zhang, M. & Kornberg, R.D. Histone octamer transfer by a chromatin-remodeling complex. Cell 96, 389–392 (1999).
42. Mendenhall, E.M. et al. GC-rich sequence elements recruit PRC2 in mammalian ES cells. PLoS Genet. 6, e1001244 (2010).
43. Stanton, B.Z. et al. Smarca4 ATPase mutations disrupt direct eviction of PRC1 from chromatin. Nat. Genet. http://dx.doi.org/10.1038/ng.3735 (2016).
44. Deal, R.B., Henikoff, J.G. & Henikoff, S. Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. Science 328, 1161–1164 (2010).
45. Buenrostro, J.D., Giresi, P.G., Zaba, L.C., Chang, H.Y. & Greenleaf, W.J. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat. Methods 10, 1213–1218 (2013).
46. Jadhav, U. et al. Acquired tissue-specific promoter bivalency is a basis for PRC2 necessity in adult cells. Cell 165, 1389–1400 (2016).
47. Schnitzler, G., Sif, S. & Kingston, R.E. Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. Cell 94, 17–27 (1998).
48. Ronan, J.L., Wu, W. & Crabtree, G.R. From neural development to cognition: unexpected roles for chromatin. Nat. Rev. Genet. 14, 347–359 (2013).
49. Khavari, P.A., Peterson, C.L., Tamkun, J.W., Mendel, D.B. & Crabtree, G.R. BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. Nature 366, 170–174 (1993).
50. Narayanan, R. et al. Loss of BAF (mSWI/SNF) complexes causes global transcriptional and chromatin state changes in forebrain development. Cell Rep. 13, 1842–1854 (2015).
51. Yoo, A.S., Staahl, B.T., Chen, L. & Crabtree, G.R. MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature 460, 642–646 (2009).
52. Yoo, A.S. et al. MicroRNA-mediated conversion of human fibroblasts to neurons. Nature 476, 228–231 (2011).
53. Victor, M.B. et al. Generation of human striatal neurons by microRNA-dependent direct conversion of fibroblasts. Neuron 84, 311–323 (2014).
54. Singhal, N. et al. Chromatin-remodelling components of the BAF complex facilitate reprogramming. Cell 141, 943–955 (2010).
55. Weinberg, P., Flames, N., Sawa, H., Garriga, G. & Hobert, O. The SWI/SNF chromatin remodeling complex selectively affects multiple aspects of serotonergic neuron differentiation. Genetics 194, 189–198 (2013).
56. Dawson, M.A. & Kouzarides, T. Cancer epigenetics: from mechanism to therapy. Cell 150, 12–27 (2012).
ONLINE METHODS
Cells and construct design. CiAO MEFs containing a modified Pou5f1 promoter (with 12× ZFHD1 and 6× GAL4 sites upstream of the promoter) were generated, cultured and maintained as previously described. Briefly, lentiviral delivery constructs bearing an EEF1A1 promoter and either puromycin or blasticidin resistance were generated to contain the constructs described here (Supplementary Fig. 1b). To generate recruitable forms of BAF complexes, genes encoding individual BAF complex subunits (SS18, SMARCA4, SMARCB1, SMARCE1) were fused in frame to sequences encoding FRB-V5. We generated the following constructs: FRB-V5-SS18, FRB-V5-SS18-SSX1, FRB-V5-SMARCE1, and FRB-V5-SMARCB1, SMARCA4-FRB-V5, SMARCA4(K785R)-FRB-V5, and a control FRB-V5-STOP to be paired with coinfected ZFHD1-FKBP.

Recruitment assays. Briefly, adherent CiAO MEFs were treated with 3 nM (final concentration) rapamycin (sirolimus; Selleckchem, S1039) (ON experiments) or 3 nM rapamycin followed by 30 nM FK1012 (OFF/washout experiments) for prescribed times, as indicated (2.5 min–24 h). For acute time points, cells were collected rapidly by washing medium out once with PBS, scraping cells off the plates with a cell scraper, and resuspending in cell fix buffer (50 mM HEPES, 1 mM EDTA, 0.5 mM EGTA, and 100 mM NaCl) and formaldehyde-based fixing for subsequent ChIP analyses.

Immunoblot analyses. BAF complex subunits modified with FRB-V5 tags were tested for expression and complex integration using standard nuclear protein extract purification and subsequent anti-SMARCA4 immunoprecipitation from 150 µg of nuclear extract input (anti-SMARCA4 (SCBT G7 clone; sc-17796)). Immunoblot analyses were performed using the antibodies indicated: SMARCB1 (SCBT clone A-5; sc-166165), SMARCE1 (Bethyl, A300-810A), SS18 (SCBT clone H80; sc-28698), V5 epitope tag antibody (Invitrogen, R960-25).

Chromatin immunoprecipitation. Briefly, for rapid time course assays, adherent CiAO MEFs were washed once in PBS, scraped off plates into fix buffer (50 mM HEPES, 1 mM EDTA, 0.5 mM EGTA, and 100 mM NaCl), resuspended, and immediately fixed in formaldehyde (for 10 min at room temperature). After quenching cross-linking using 0.125 M (final) glycine, cells (7–10 × 10^6) were washed and sonicated for 13.5 min using a Covaris E220 sonicator. Chromatin input was reverse cross-linked and evaluated for shearing efficiency. 100–150 µg of chromatin stock was used per immunoprecipitation reaction. Antibodies (3 µg/ChIP reaction) used for ChIP (listed in Supplementary Table 1) were incubated with chromatin stock and Protein G Dynabeads (1004D) overnight at 4 °C. Following washing, immunoprecipitated material was eluted and subjected to reverse cross-linking. Finally, DNA precipitation was performed using phenol/chloroform extraction and ChIP DNA was reconstituted in 50 µl of TE for qPCR reactions. The sequences for the primers used in ChIP assays are listed in Supplementary Table 2.

ChIP analysis and statistical calculations. CiA knock-in locus-specific primers were generated and are reflected in Supplementary Table 2 with plus (+) and minus (−) direction distances calculated from the middle of the ZFHD1 recruitment domain as well as minus (+/−) distances calculated from the Pou5f1 TSS. Briefly, enrichment (bound over input) values were normalized to values with no rapamycin treatment. Standard deviation was calculated over n = 3 repeat experiments for each primer set. Student’s two-sample unpaired t tests were performed to determine statistical significance.

Tn5 transposase chromatin accessibility assays (ATAC–qPCR). Following various recruitment conditions, 5 × 10^4 CiAO MEFs were collected, washed once in PBS, washed once in RSB buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2), and centrifuged at 500g for 5 min at 4 °C. Cells were then lysed in lysis buffer (500 µl of RSB buffer + 5 µl of 10% NP-40) for 5 min on ice, spun at 500g for 5 min, resuspended in Tagment DNA/Enzyme Buffer Mix (Illumina Nextera Sample Preparation Kit, FC-121-1030), and incubated for 30 min at 37 °C. Following Tn5 transposase enzyme reaction, DNA was purified using the Qiagen MinElute PCR Purification kit (28004). Transposed DNA fragments were amplified via qPCR to the appropriate number of cycles, and the library was purified using a Qiagen PCR Cleanup kit eluted in 20 µl of elution buffer (10 mM Tris buffer, pH 8.0). CiA locus-specific qPCR was performed using the primers in Supplementary Table 2.