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Control of stimulus-dependent responses in macrophages by SWI/SNF chromatin remodeling complexes

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Abstract

Epigenetic regulation plays an important role in controlling the activation, timing, and resolution of innate immune responses in macrophages. Previously, SWI/SNF chromatin remodeling was found to define the kinetics and selectivity of gene activation in response to microbial ligands; however, these studies do not reflect a comprehensive understanding of SWI/SNF complex regulation. In 2018, a new variant of the SWI/SNF complex was identified with unknown function in inflammatory gene regulation. Here, we summarize the biochemical and genomic properties of SWI/SNF complex variants, and the potential for increased regulatory control of innate immune transcriptional programs in light of such biochemical diversity. Finally, we review the development of SWI/SNF complex chemical inhibitors and degraders that could be used to modulate immune responses.

In response to microbial ligands and cytokines, macrophages induce a cascade of events resulting in a transcriptional response important for macrophage activation, cytokine release, and the adaptive immune response. This highly coordinated program is executed by stimulus-regulated transcription factors (SRTFs) [1]. The binding and activity of SRTFs is in turn restricted to cognate binding sites within ‘cis-regulatory elements’ made accessible by the action of myeloid lineage-determining transcription factors (LDTFs) working in conjunction with epigenetic regulators (Box 1) [1]. Epigenetic regulators can additionally contribute to gene-specific regulatory requirements that manifest in differences in gene induction kinetics, selectivity, and resolution. Among these, the mammalian SWI/SNF complexes exhibit genetic requirements in innate immune cell differentiation and response to inflammation [2–5]. SWI/SNF complexes are a family of polymorphic ATP-dependent chromatin remodeling complexes assembled around a core ATPase, either BRG1 or BRM. These macromolecular machines are thought to utilize energy derived from ATP hydrolysis to remodel nucleosomes on chromatin to create accessible regions [6]. Indeed, SWI/SNF complexes are recruited to cis-regulatory elements such as promoters and enhancers, where they contribute to the establishment and maintenance of chromatin accessibility at transcription factor (TF) binding sites [2, 7–12]. Up until 2018, the SWI/SNF complex was

thought to exist as two main variants: the ARID1A-containing BAF complex (BAF) and the Polybromo-associated BAF (PBAF) complex. The recent discovery of the bromodomain-containing protein 9 (BRD9)-associated BAF complex, also known as the ncBAF or non-canonical BAF complex, provides further evidence of compositional heterogeneity within the SWI/SNF complexes in mice and humans (Figure 1) [13–16]. We and others have shown that SWI/SNF complex subunits contribute to complex targeting and function through domains that mediate specific interactions with binding partners, TFs, or features on chromatin [5, 14, 15, 17, 18]. Thus, we propose that biochemical heterogeneity gives rise to functionally specific properties of individual SWI/SNF complex variants, which provides greater regulatory control over transcriptional networks. Further, the use of newly developed epigenetic inhibitors targeting subunits unique to a particular SWI/SNF complex variant is likely to have specific therapeutic utility. In this Review, we revisit previous studies exploring the requirement for SWI/SNF complexes in macrophage responses to inflammatory stimuli and discuss the potential integration of SWI/SNF complex functions in inflammatory gene activation in light of these recent advances.

Requirement for SWI/SNF complex remodeling in inflammatory gene activation

Primary versus Secondary Response Genes

Upon activation of Toll-like receptor 4 (TLR4) with lipopolysaccharide (LPS) treatment, mammalian macrophages upregulate pro-inflammatory genes, which can be divided into primary response genes (PRGs) and secondary response genes (SRGs) based on the requirement for new protein synthesis [19]. PRGs can be further divided into early and late PRGs by their activation kinetics. Previous studies showed that siRNA knockdown of the shared ATPases BRG1 and BRM in bone marrow derived macrophages (BMDMs; always murine in this review) or expression of an enzymatically dead BRG1 variant suppressed transcriptional activation of SRG genes *Il6*, *Il12b* and late PRG genes *Ccl5* and *Saa3,* without affecting early PRG genes *Tnf*, *Cxcl2*, and *Ptgs2* [3]. In addition, relative to unstimulated BMDMs, LPS induced an increase in chromatin accessibility at promoter and enhancer regions of SRGs and late PRGs, which was dampened by disruption of both SWI/SNF complex ATPase subunits [3]. These results highlighted the requirement for BRG1/BRM-dependent chromatin remodeling for SRG induction (Figure 1). In contrast, many PRGs were found to harbor features of active chromatin in resting macrophages, including DNaseI hypersensitivity and RNA Polymerase II binding, consistent with rapid activation of PRGs following stimulation [20–23]. The requirement for chromatin remodeling at SRGs and late PRGs thus provides an additional layer of regulation that can contribute to slower and more selective gene induction in response to various inflammatory signals [20, 21].

SWI/SNF Complex recruitment by Transcription Factors

The mechanism by which SWI/SNF complexes selectively regulate specific gene classes during inflammatory stimulation is not known. However, evidence from multiple systems suggests that a genetic requirement for SWI/SNF subunits can be mechanistically attributed

to a requirement for chromatin remodeling at TF binding sites [2, 4, 5, 7, 8, 11, 12, 24, 25]. TFs likely recruit SWI/SNF complexes to sequence-specific sites on chromatin, although the interdependent relationship between SWI/SNF complex binding and TF binding in many contexts makes order of recruitment events difficult to establish [8]. Stimulus-dependent transcription in macrophages is defined by a regulatory network of LDTFs working in combination with SRTFs and general TFs [1] (Box 1). Put simply, myeloid-specific LDTFs such as PU.1, C/EBP-β, and IRF8 act during differentiation to create open chromatin at cell type-specific enhancers [1]. In this way, LDTFs define the enhancer repertoire accessible for SRTF binding, thereby specifying the transcriptional program that is induced in response to stimulation. We thus hypothesize that SWI/SNF complex interaction with LDTFs and SRTFs is likely to contribute to SWI/SNF-dependent epigenetic changes that are required for inflammatory gene induction (Figure 2). In support of a potential role for SWI/SNF complexes at LDTF binding sites, conditional deletion of ARID1A in hematopoietic cells following polyI:C injection into *Arid1a*$^{f/f}$*;Mx1-Cre* mice resulted in impaired hematopoietic differentiation due to loss of chromatin accessibility at sites that contained motifs for LDTFs PU.1, Runx1, Gata and C/EBP-α [2]. Furthermore, SWI/SNF complexes were found to be actively recruited by LDTF PU.1 following tamoxifen-induced nuclear translocation of a PU.1-Estrogen Receptor (PUER) fusion protein, exogenously expressed in a PU.1$^{-/-}$ cell line derived from fetal liver hematopoietic progenitors of a PU.1$^{-/-}$ mouse [26]. SWI/SNF complex recruitment by LDTFs could thus contribute to signal-dependent responses through the establishment of accessibility at myeloid-specific enhancers, as well as at *de novo* enhancers activated in response to stimulation through cooperative interactions between LDTFs and SRTFs [27–29]. In this way, SWI/SNF complexes working in cooperation with LDTFs during hematopoietic development could play an important role in establishing stimulus-dependent responses.

Following stimulation and in response to SRTFs, SWI/SNF complexes likely relocalize to sites of *de novo* and increased chromatin accessibility correlated with gene induction. In the context of TLR or cytokine stimulation, SRTFs include members of the NF-κB, AP-1, STAT and IRF families [1]. Upon LPS treatment of BMDMs, nucleosome remodeling at the SRG *Il12b* was found to be dependent on protein synthesis, implying that the recruitment of SWI/SNF complex-dependent remodeling activity to SRGs is mediated by proteins that are induced and translated during the primary response to stimulation [3]. This includes cytokines induced during the primary response that result in autocrine signaling and activation of constitutively expressed SRTFs. For example, many TLR4-induced SRGs are dependent on Interferon alpha Receptor (IFNαR) signaling activated in response to *Ifnb* induction in BMDMs [30]. The fact that BRG1 physically associates with STAT1 [31] and STAT2 [32], and is required for remodeling of a subset of interferon-stimulated genes (ISGs) in the context of IFN-α/β and IFN-γ stimulation [31–33] could thus explain the requirement for BRG1 in the induction of many TLR4-induced SRGs activated in response to IFNαR signaling. Induced SRTFs (iSRTFs) encoded by PRGs can also regulate SWI/SNF complex recruitment to SRGs. For example, the iSRTF IκBζ encoded by the PRG *Nfkbiz* was shown to be involved in BRG1 complex recruitment to the *Il6* and *Il12* promoters upon LPS stimulation of BMDMs via the NFκBp50-IκBζ-Akirin2-BAF60 axis [34–36]. This function appears to be conserved across species as Akirin is required for the induction of a subset of

LPS- and IL-1-inducible genes in *Drosophila melanogaster* S2 cells through direct association of the SWI/SNF BAF60 subunit homolog BAP60 with Akirin [37]. Finally, the PRG *lincRNA-Cox2* encoding a non-coding RNA (ncRNA) was found to be recruited to the promoters of late PRGs *Ccl5* and *Saa3* in response to TLR4 stimulation of BMDMs, and siRNA knockdown of *lincRNA-Cox2* abolished the activation of those genes, as well as BRG1 recruitment to the *Ccl5* and *Saa3* promoters and accompanying associated chromatin changes [38]. These data suggest that there are multiple mechanisms by which SWI/SNF complexes can participate in stimulus-dependent responses through engagement with LDTFs as well as stimulus-responsive and induced proteins or RNAs.

These data provide an intuitive framework for the role of SWI/SNF complexes in stimulus-dependent responses. However, it bears noting that a comprehensive study of SWI/SNF complex requirement in inflammatory gene induction is currently lacking. Genomic analyses are likely to shed light on gene-specific regulatory requirements that cannot be fully explained by what previous studies have shown on a limited number of genes. Furthermore, we lack a systems-level view of how SWI/SNF complexes collaborate with transcription factors to execute stimulus-dependent transcription. Previous studies referenced above suggest that SWI/SNF complexes can be recruited by LDTFs, as well as constitutively expressed SRTFs, iSRTFs, and ncRNAs activated in response to TLR or autocrine IFNαR signaling. Elucidation of regulatory relationships between SWI/SNF complexes and TFs, as well as RNA Polymerase II and other epigenetic regulators, will thus be instrumental in understanding gene-specific requirements for SWI/SNF complex remodeling in response to inflammatory stimulation in macrophages.

New kid on the block: the BRD9-containing ncBAF complex

In 2018, we and others discovered that there is a smaller, non-canonical SWI/SNF complex, also known as the ncBAF complex that exists alongside the BAF and PBAF complexes (Figure 1) [13–16]. Mass spectrometric studies from mouse and human cells determined that the ncBAF complex contains the ATPase subunit BRG1 and the core subunits BAF155 and BAF60A, and that it uniquely incorporates the bromodomain containing protein 9 (BRD9) and the glioma tumor suppressor candidate region 1 (GLTSCR1) or a paralog, GLTSCR1L. Furthermore, glycerol gradient sedimentation analyses and immunodepletion experiments demonstrated that BRD9, similar to known BAF and PBAF subunits, is a dedicated subunit of the ncBAF complex [14, 15, 39] and CRISPR-Cas9 mediated deletion of BRD9 results in loss of ncBAF complex integrity in human cancer cell lines by glycerol gradient sedimentation analyses [16]; these data suggest that the function of BRD9 in most cell types can be attributed to its role in ncBAF complexes. The ncBAF complex is distinct as it lacks BAF-specific subunits ARID1A/ARID1B and BAF45D and the PBAF-specific subunits ARID2, PBRM1, BRD7 and BAF45A (Figure 1) [13–16]. The ncBAF complex also does not contain BAF47 and BAF57, subunits that are shared between the BAF and PBAF complexes [13–16]. The specific composition of each SWI/SNF complex assembly provides diverse functionalities in the form of protein domains that bind distinct chromatin features, TFs, or other epigenetic regulators. Indeed, many SWI/SNF subunits contain evolutionarily conserved domains that can bind to non-specific DNA sequences or post-translationally modified residues on histones (e.g. bromodomains) (Box 2) [6]. An important outcome of

this compositional heterogeneity is the potential for distinct targeting and functional interactions of different SWI/SNF complex assemblies, which can contribute to specific requirements in transcriptional responses. Below we review recent advances in our understanding of the localization and function of SWI/SNF complex variants, highlighting several novel mechanisms that could be operational in the setting of inflammatory stimulation (Figure 3).

SWI/SNF complex localization and function

Enhancer Regulation by BAF complexes

Using antibodies that recognize ARID1A or BAF45D, BAF complexes have been shown to be enriched at poised, active, and super enhancers, while ncBAF and PBAF complexes are more commonly found at promoters in mouse and human cells (Box 1) [14, 15, 18]. These data suggest that BAF complexes are important for establishing or maintaining features of enhancer architecture. Indeed, we and others have shown that loss of ARID1A, BRG1, or BAF47 results in loss of chromatin accessibility at enhancers by Assay for Transposon-Accessible Chromatin followed by genome-wide sequencing (ATAC-seq), resulting in nucleosome occlusion of transcription factor binding sites and loss of transcription factor binding by ChIP-seq in mouse and human cells [8–12, 40–43]. Other aspects of enhancer architecture are also affected upon BAF complex disruption, including loss of H3K4me and H3K27ac due to a direct association of BAF complexes with the H3K27ac histone acetyltransferase p300 in mouse embryonic fibroblasts and human cancer cell lines [44]. This activity underlies the requirement for BAF subunits in the development of many cellular lineages, including hematopoietic lineages (Table 1), and may also contribute to the activation and *de novo* creation of enhancers during stimulus-dependent responses (see Outstanding Questions). Furthermore, a direct interaction between BAF complexes and AP-1 members has been observed in human neural stem cells [7, 24], and BAF complexes are required for accessibility and H3K27ac at AP-1 binding sites in several human cell types [9, 42, 43]. Given that AP-1 members can collaborate with LDTFs in the establishment of cell type-specific enhancers [24, 45] and act in a stimulus-responsive way [28, 46, 47], this could be a general mechanism by which BAF complexes are recruited to enhancers to prime and activate certain loci in response to stimulation. Collectively, these data indicate that a key function of the BAF complex is to establish and maintain enhancer chromatin architecture in response to TF recruitment and to stabilize TF binding.

Gene repression through association of BAF complexes with histone deacetylases

ARID1A has also been observed to bind promoters, where it primarily acts as a repressor through interactions with histone deacetylases [48, 49]. This regulation is particularly relevant in the context of ARID1A loss-of-function mutations in ovarian cancer where ARID1A loss leads to de-repression of cytokine gene expression. Specifically, in a genetically engineered mouse model (GEMM) of ovarian cancer, simultaneous conditional deletion of ARID1A and activation of the Phosphatidylinositol 3-Kinase p110 alpha mutant allele (PIK3CA H1047R) following Adenovirus-Cre injection into the ovarian bursa of *Arid1a*$^{fl/fl}$;*(Gt)Rosa26Pik3ca*^{*H1047R} mice resulted in the development of ovarian tumors with increased expression of *Il6* relative to control ovaries; this was due to the fact that *Il6* is

normally repressed when ARID1A binds to the *Il6* promoter [48]. Similarly, knockdown of ARID1A in an immortalized normal human ovarian epithelial cell line expressing PIK3CA gain-of-function alterations resulted in the induction of cytokine genes such as *IL1A*, *IL1B*, *CXCL1*, *CXCL8*, *IL6*, and *IL8* [49]. In this context, knockdown of ARID1A resulted in loss of ARID1A binding to *IL6* and *IL8* promoters, with concomitant loss of the corepressor mSin3A and HDAC1 binding relative to WT cells [49]. Of relevance, HDAC1 and HDAC3 are bound to many inducible genes in unstimulated murine BMDMs in the context of NCoR corepressor binding [20, 50], and SWI/SNF subunits BRG1, BAF170, BAF155, and BAF47 associate with the NCoR corepressor complex [51]. SWI/SNF complexes may thus repress inducible genes through association with co-repressors such as NCoR, mSin3a, and/or HDAC1/3. The elucidation of a SWI/SNF-dependent mechanism of gene repression could provide important insights into basal control of inducible genes as well as the resolution of stimulus-dependent inflammatory responses. In addition, the generalizable nature of this mechanism across cell types has important implications for disease settings in which inflammatory cytokines are aberrantly upregulated, for example in cancers.

Bromodomain-dependent recruitment of ncBAF complexes

Reader domains in SWI/SNF complex subunits have long been assumed to have a role in complex targeting or chromatin binding affinity. The recent development of targeted inhibitors in conjunction with genetic and CRISPR-Cas9-guided domain interrogation [52] has been instrumental in identifying the contribution of such functional domains to SWI/SNF complex targeting and activity on chromatin. Several recent studies have described the development of potent and selective small-molecule inhibitors of the BRD9 bromodomain, which interfere with bromodomain recognition of acetylated lysines [53–55]. Treatment with the BRD9 bromodomain inhibitors I-BRD9 or BI-7273 reduces BRD9 binding by ChIP-seq in mouse embryonic stem cells and human synovial sarcoma cell lines [14, 56], suggesting that the BRD9 bromodomain contributes to targeting ncBAF complexes. In addition, I-BRD9 was shown to enhance the binding of the PBAF-specific subunit bromodomain containing protein 7 (BRD7) to the acetylated lysine 91 (K91) residue of the Vitamin D receptor when transfected into human 293T cells [57]. In the presence of Vitamin D ligand, VDR binding to PBAF complexes is favored and VDR-dependent transcription is promoted in the rat pancreatic INS-1 cell line, an effect that can be enhanced by co-treating with I-BRD9 [57]. It is not known what fraction of ncBAF and PBAF complexes are targeted in this manner or whether the dynamic association of ncBAF and PBAF complexes via competitive binding of BRD9 and BRD7 to acetylated transcription factors is a general phenomenon; however, because nuclear receptors control many different aspects of mammalian physiology, including inflammation, the conservation of K91 among other nuclear receptors suggests this could be an important mechanism of SWI/SNF complex cross-talk in multiple systems.

Cooperative regulation of ncBAF complexes in association with BRD4

The ncBAF complex also interacts with the BET (bromodomain and extra terminal domain) protein BRD4 via interactions with GLTSCR1 [58] in human 293T cells and BRD9 in mouse embryonic stem cells (mESC) and human cancer cell lines [13, 14]. Indeed, BRD9 and BRD4 have a high degree of co-localization on the genome and pharmacological

inhibition of BRD4 binding to chromatin via treatment with the BET bromodomain inhibitor (BETi) JQ1 evicts both BRD4 and BRD9 in mESCs [14]. This interaction underlies BRD9:BRD4 co-regulation of a common set of genes in mESCs, including *Nanog* and *Prdm14*. In BMDMs, BRD4 has been shown to promote the inflammatory response elicited by LPS. Relative to vehicle-treated controls, both conditional deletion of *Brd4* following tamoxifen administration to *Brd4*$^{fl/fl}$*;UBC:ER*^{T2}*-Cre* BMDMs or BETi treatment of BMDMs resulted in reduced expression of *Il6* and *Il10* in response to LPS stimulation, along with a subset of other LPS-induced genes; this was due to impaired recruitment of P-TEFb and RNA Polymerase II to stimulus-responsive promoters following LPS addition by ChIP-seq [59–61]. As a result, BETi treatment protected mice from LPS-induced or heat-killed *Salmonella typhimurium*-induced endotoxic shock [60, 61] and death after cecal ligation and puncture (CLP) [60]. Similarly, treatment with JQ1 resulted in reduced expression of pro-inflammatory genes *Sele*, *Vcam*, and *Ccl2* induced by TNFα in endothelial cells and protection from atherosclerosis in LDL receptor-deficient (*Ldlr−/−*) mice fed an atherogenic cholesterol-rich diet relative to vehicle-treated animals [62]. Mechanistically, this was attributed to a requirement for BRD4 at *de novo* super enhancers established by NF-κB upon TNFα-stimulation because JQ1 treatment blocked the recruitment of RNA Polymerase II to TNFα-induced super enhancers and their associated genes as well as transcription of these genes following TNFα-stimulation [62]. Given the association between ncBAF complexes and BRD4, these data suggest that ncBAF complexes could be functioning in inducible transcription through regulation of one or more BRD4-dependent processes; this is is relevant as it might guide the use of BRD9 inhibitors in inflammation-associated diseases such as sepsis and atherosclerosis

ncBAF-specific localization to topologically-associated boundaries

ChIP-seq for BRD9 shows that ncBAF complex binding is additionally strongly enriched at binding sites for CTCF and Cohesin, proteins known for their roles in regulating chromatin organization in mouse and human cells [63]. Indeed, BRD9, but not ARID1A, localize to topologically associating domain (TAD) boundaries in mESCs [14]. Thus, the ncBAF complex could be cooperating with these structural proteins for the formation and/or maintenance of chromatin loops, shown to be critical in macrophage development and in response to stimulation [64]. Specifically, inducible deletion of the cohesin subunit *Rad21* following tamoxifen administration into *Rad21*$^{fl/fl}$*;Rosa26-ER*^{t2}*-Cre* BMDMs resulted in reduced inflammatory gene expression due to failed activation of inducible enhancers and local chromatin looping in response to LPS [64]. Inducible *Brd9* deletion and/or acute chemical degradation of BRD9 will similarly be useful in establishing the potential cooperation between the ncBAF complex and CTCF/Cohesin in TLR- and cytokine-induced chromatin looping events in macrophages. Because the binding to CTCF/Cohesin sites appears to be a specific property of the ncBAF complex and not other SWI/SNF complex variants, the identification of a potential regulatory interaction between ncBAF complexes and CTCF/Cohesin might provide a molecular for the specific role of ncBAF complexes in transcriptional regulation.

Integration of SWI/SNF complex functions

Biochemical diversity among SWI/SNF complexes and the array of functionalities that this affords gives the advantage of greater regulatory control over gene expression programs. In mESCs, the ncBAF complex cooperates with transcription factors Sp5 and Kruppel like factor 4 (KLF4) and the epigenetic reader BRD4 to regulate the stimulus-dependent program of naïve pluripotency [14]. This function is not shared by the BAF complex, which regulates chromatin accessibility at sites bound by LDTFs OCT4, SOX2, and NANOG in mESCs [8, 10]. Thus, the BAF and ncBAF complexes are both integrated into the mouse pluripotency transcriptional network, but they regulate distinct sets of genes. In the same way, the identification of specific relationships between SWI/SNF complexes and TFs is likely to provide mechanistic basis for differential requirements of SWI/SNF complex subunits in inflammatory gene induction. There is strong evidence now from multiple systems, including mESCs, that BAF complex interactions with LDTFs are critical for enhancer selection and activity [8, 10]. Thus, we posit that BAF complexes working in collaboration with myeloid LDTFs may set the stage for stimulus-responsive events dependent on BAF, ncBAF and/or PBAF complexes in association with SRTFs. Furthermore, in the context of IFN-γ or IL-4 stimulation of BMDMs, the activation of latent enhancers by LDTFs working in collaboration with SRTFs results in cellular memory defined by the maintenance of active enhancers even after the removal of the stimulus, allowing a more rapid gene activation upon restimulation [27, 65]. The involvement of the SWI/SNF complex in establishing such epigenetic bookmarks could thus have important implications for immune gene activation *in vivo*, which typically involves the integration of multiple signals.

Pharmacological Targeting of SWI/SNF Complexes

The potential role for SWI/SNF complex inflammatory responses makes it an attractive target for drug development; however, the biochemical properties of these large, multi-subunit complexes have made development of small molecule inhibitors particularly challenging. The development of bromodomain inhibitors directed against various bromodomain-containing SWI/SNF subunits has allowed for the first time, the ability to probe the function of these domains in SWI/SNF complex targeting, assembly, and activity (Figure 4, Table 2). Additionally, the extension of this chemistry through the development of proteolysis targeting chimeras (PROTACs) that link bromodomain binding domains to chemical moieties that recruit the E3 ligases VHL or cereblon, has brought us the first chemical degraders directed against SWI/SNF subunits [66]. Work from our group and others has demonstrated that treatment with BRD9 bromodomain inhibitors (BRD9i) -- including I-BRD9 and BI-7273 --is associated with BRD9-specific functions including maintaining naïve pluripotency of mESCs [14], cancer cell line growth (associated with *Myc* expression) in acute myeloid leukemia [53], as well as Vitamin D-dependent control of pancreatic β cells [57]. dBRD9 was additionally found to be effective at slowing the growth of malignant rhabdoid tumor (MRT) and synovial sarcoma (SS) cell lines in vitro [15, 16, 56] and in vivo xenografts [56], consistent with RNAi and CRISPR-Cas9 screen results reporting BRD9 dependencies in these cell lines due to driver alterations in BAF subunits [67, 68]. Similarly, the BRG1/BRM/PBRM1 PROTAC ACBI1 is a potent inhibitor of cell growth in BRG1-deficient human cancer cell lines dependent on BRM [69]. Finally, a large

scale screening effort based on reporter activation in mESCs has aided the identification of a series of SWI/SNF complex inhibitors with activity in reversing HIV-1 latency in primary human $CD4^+$ T cells and patient samples [70, 71] as well as slowing the growth of cancer cell lines [72]. In particular, a number of 12-membered macrolactams were identified from these studies, several of which bind ARID1A-containing BAF complexes and phenocopy the effects of ARID1A deletion in reactivating HIV-1 gene expression in primary human $CD4^+$T cells and mESC transcription [70]. ARID1A inhibitors such as BRD9 inhibitors/degraders, are predicted to act selectively based on the specific incorporation of ARID1A and BRD9 into BAF and ncBAF complexes, respectively. Thus, the development of such compounds is an important advance towards being able to potentially modulate the activity of specific SWI/SNF assemblies in therapeutic settings.

Translational Outlook

A mechanistic understanding of the role of SWI/SNF complexes in stimulus-dependent responses in BMDMs can lay the groundwork for a deeper understanding of the epigenetic processes that govern macrophage responses during inflammation and immune-related diseases. Indeed, macrophages can acquire different functional programs in response to inflammatory stimuli that can contribute to protection or exacerbation of disease in different contexts, including infection, sepsis, obesity, atherosclerosis, and cancer. In response to an initial stimulation with LPS or endotoxins, macrophages become refractory to secondary stimulation, known as LPS tolerance, which resembles the functional state of macrophages during sepsis [73]. However, in response to infection or vaccination (e.g. *Candida albicans* infection, Bacille Calmette-Guérin (BCG) or measles vaccinations), macrophages acquire long-term responsiveness and provide protection to secondary infections, referred to as ‘trained immunity’ [74, 75]. We and others have shown that gene-specific epigenetic features, including the recruitment of SWI/SNF complexes, correlate with the transcriptional programs that define the refractory and hyper-responsive states of macrophages in response to LPS or β-glucan -- stimuli that mimic tolerance and trained immunity, respectively [75–77]. The identification of epigenetic mechanisms that fine-tune inflammatory responses or provide gene-specific regulation of different functional aspects of the immune response could thus be relevant to macrophage responses in immune-related diseases. Future experiments geared towards understanding the role of SWI/SNF complexes and other epigenetic regulators in macrophage dysfunction in vivo can help determine the potential therapeutic efficacy of epigenetic modulation of macrophage responses in these contexts.

Concluding Remarks

Previous studies demonstrated a requirement for SWI/SNF complexes in inflammatory gene induction in macrophages, but failed to delineate subunit-specific functions associated with SWI/SNF complex variants (Outstanding Questions). Gaining insight into the potentially diverse roles of the SWI/SNF complex variants in stimulus-dependent transcription requires ChIP-seq of variant-specific subunit binding in resting and stimulated macrophages, as well as genome-wide profiling of nascent transcription and chromatin accessibility following SWI/SNF subunit deletion. Historically, the ability to perform ChIP-seq on SWI/SNF complex subunits was hampered by the dearth of high-quality antibodies and the sensitivity

of these complexes to fragmentation during sonication. However, high-quality mapping of certain subunits has emerged over the last five years, which makes these studies potentially feasible in macrophages. Additional technical hurdles include the requirement for these complexes in myeloid and lymphoid differentiation [2, 78, 79], which precludes experiments directed towards understanding the role of SWI/SNF complexes in inflammatory responses in mature macrophages. Inducible genetic knockouts may be helpful in this respect, or alternatively, the development of degron-tagged alleles that allow for rapid degradation in response to chemical addition. In addition, SWI/SNF subunit inhibitors and PROTACs can be used, which allows for acute and reversible inhibition of these complexes. Indeed, such compounds can be invaluable in teasing out the role of SWI/SNF complexes in primary versus secondary stimulation in the context of LPS tolerance, innate immune adaptation, and trained immunity. Future studies in this vein will help define the contribution of SWI/SNF complexes to innate immune gene activation and provide a general framework for the integration of SWI/SNF complex functions in stimulus-dependent responses.

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Glossary

Akirin
highly conserved 201-residue protein with orthologs identified throughout metazoa; functions as a transcriptional cofactor

Assay for Transposon-Accessible Chromatin followed by genome-wide sequencing (ATAC-seq)
technique to assay chromatin accessibility; uses the Tn5 transposase to deposit DNA adapters into open chromatin, which are then amplified and sequenced by next-generation sequencing

CRISPR-Cas9
prokaryotic immune system conferring resistance to foreign genetic elements such as those present within plasmids and phages by Cas9-mediated cleavage of DNA sequences complementary to the CRISPR sequence. Developed into a technology to edit DNA sequences within organisms

Bone Marrow Derived Macrophages (BMDMs)
macrophages harvested from mouse bone marrow and differentiated in cytokine (e.g., M-CSF) containing media

Bromodomain
evolutionarily conserved domain that specifically binds to acetylated lysines on histones and non-histone proteins

Chromatin loops
created by interactions between non-adjacent DNA sequences

Cohesin
multi-subunit protein complex with ATPase activity composed of four proteins; forms a ring around DNA strands --important during mitosis and meiosis to keep sister chromatids held together and to facilitate interactions between non-adjacent DNA sequences

CTCF
highly conserved zinc finger protein (aka 11-zinc finger protein or CCCTC-binding factor) involved in the regulation of chromatin architecture

Degron-tagged alleles
allele created by fusing a degron (or protein domain) regulating protein degradation rates, to a gene of interest. Examples: the auxin-inducible system, allowing rapid degradation of proteins of interest fused to the Auxin-inducible degron (AID) domain in the presence of the auxin responsive F-box protein, TIR1; this forms a functional SCF^{TIR1} ubiquitin ligase with the endogenous subunits conserved in all eukaryotic cells

Enhancers
distal regulatory sites regulating cell type-specific gene expression

Epigenetic Readers
proteins that bind post-translational modifications on histones or other proteins bound to chromatin though specific recognition domains

Lineage-determining transcription factors (LDTFs)
TFs that specify a particular cell fate

Macrolactams
organic chemistry term -- compounds that are macrocyclic (having a closed ring of more than twelve atoms) lactams (any class of cyclic amides)

NCoR corepressor
protein bearing many nuclear receptor interaction domains; known to recruit histone deacetylases to DNA promoters

Pioneer Factors
TFs with an ability to bind cognate sites on nucleosomal DNA

Primary Response Genes (PRGs)
pro-inflammatory genes which are rapidly transcribed following LPS stimulation in macrophages and do not require new protein synthesis

Proteolysis targeting chimeras (PROTACs)
heterobifunctional small molecules that bind a target protein and induce target protein degradation via recruitment of an E3 ligase such as VHL or cereblon

Secondary Response Genes (SRGs)
pro-inflammatory genes whose induction requires new protein synthesis following TLR4 stimulation

Stimulus-regulated transcription factors (SRTFs)
TFs whose activity is regulated by stimulation

SWI/SNF complex
multi-subunit protein complex; uses energy derived from ATP hydrolysis to displace or move nucleosomes to regulate chromatin accessibility

Tolerance
state in which macrophages become refractory to stimulation after an initial exposure to LPS or endotoxins, which often occurs in sepsis and is characterized by an inability to produce pro-inflammatory cytokines

Trained Immunity
Long-term hyper-responsiveness of innate immune cells, including monocytes, macrophages, and NK cells in response to infection or vaccination, e.g. *Candida albicans* infection, Bacille Calmette-Guérin (BCG), or measles vaccination; mediated by epigenetic, metabolic, and phenotypic alterations conferring non-specific resistance to secondary infections

Toll-like receptors
family of cell surface receptors that recognize ligands derived from microbes such as LPS

Topologically-associated domains
DNA region whose sequences preferentially interact with each other (as observed from chromosome conformation capture techniques such as Hi-C); these sequences are insulated from interactions with DNA sequences in adjacent domains

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Box 1:

Cis-regulatory elements

Cis-regulatory elements are defined as regions of DNA that regulate the expression of nearby genes through the binding of trans-acting factors. Cis-regulatory elements can be classified into promoters and enhancers based in part, on the presence of various epigenetic features, including post-translational modifications (PTMs) on histone tails. Promoters are associated with histone H3 lysine 4 trimethylation (H3K4me3), whereas enhancers are enriched for H3 lysine 4 monomethylation (H3K4me). These regions of chromatin are accessible, as assessed by either DNaseI hypersensitivity or Assay for Transposon-Accessible Chromatin followed by genome-wide sequencing (ATAC-seq). Enhancers can reside within introns, immediately downstream or upstream, or several kilobases to a megabase away from the genes that they regulate. Additionally, enhancers can be further classified into poised, active, and super enhancers. Active and super enhancers can be differentiated from poised enhancers by the presence of another mark, acetylated lysine 27 on H3 (H3K27ac). Super enhancers are large domains of chromatin where clusters of active enhancers are found [80]. Like active enhancers, super enhancers are typically bound by many transcription factors, along with positive regulators of transcription, including H3K27ac histone acetyltransferases p300/CBP, the Mediator complex, and RNA Polymerase II, and exhibit enhancer RNA (eRNA) transcription.

Stimulus-dependent responses are in large part determined by the binding of transcription factors to accessible sites within cis-regulatory elements. The accessible enhancer repertoire is defined in a cell type-specific manner by lineage-determining transcription factors (LDTFs) [1]. LDTFs are often referred to as ‘pioneer factors’ because they can access their cognate sites within closed chromatin; however, epigenetic regulators, including SWI/SNF complexes, also facilitate the binding of LDTFs to chromatin. Active promoters can be ‘primed’ by pre-binding of constitutively expressed general transcription factors (GTFs). When cells encounter a stimulus, stimulus-responsive transcription factors (SRTFs) bind to motif-containing sites from the available repertoire of enhancers and promoters. SRTFs can recruit a host of additional epigenetic regulators, for example p300, thus turning poised enhancers into active enhancers. Enhancer activation promotes transcription through ‘chromatin looping’, which brings active enhancers in close proximity to the transcription start site [81]. Some stimuli also result in cooperative binding of SRTFs and LDTFs at distal sites, referred to as *de novo* or latent enhancers not previously established by LDTFs [27–29]. The activation of these sites contributes to faster and more robust gene activation in the context of restimulation and thus provides a mechanistic basis for cellular memory [27, 65].

Box 2:

Epigenetic modifications on chromatin

The basic building block of chromatin is the nucleosome, made up of 146 base pairs (bps) of DNA wrapped around an octamer of histone proteins H2A, H2B, H3 and H4. The N-terminal tails of histones are subject to various post-translational modifications (PTMs), including methylation, acetylation, phosphorylation, and ubiquitination [82]. PTMs are deposited and removed by enzymes referred to as epigenetic writers and erasers, respectively. For example, while histone acetyltransferases (HATs) catalyze the addition of an acetyl group to a lysine residue, histone deacetylases (HDACs) take this modification off [82]. PTMs contribute to the regulation of DNA accessibility in two ways: first, PTMs can directly affect the interaction between DNA and histones. For instance, acetylation neutralizes the positive charge on lysine, which reduces the affinity of the DNA to histones, making it more amenable to binding of transcription factors. Second, PTMs can recruit proteins, known as epigenetic readers, that either have enzymatic activities themselves or are associated with complexes that can further modify the chromatin landscape. For example, the bromodomain is a reader domain that binds to acetylated lysines on histone tails and other proteins [83]. The bromodomain of the BET protein BRD4 specifically interacts with acetylated histone H4 and recruits the positive transcription elongation factor, P-TEFb, which promotes RNA Polymerase II elongation activity [84]. Multiple PTMs can be found on a single histone tail, on two separate histones on the same nucleosome, or on histones belonging to two different nucleosomes. Thus, the recruitment of proteins or protein complexes depends on whether they can recognize the integrated information on chromatin. Large complexes such as the SWI/SNF complex contain multiple subunits that harbor different reader domains (e.g. bromodomains) and the sum of their activities could result in complex targeting to specific sites on chromatin.

Outstanding Questions

- Where are SWI/SNF complexes bound in resting and stimulated macrophages by ChIP-seq? How dynamic is SWI/SNF complex binding in response to stimulation? What are the specific binding properties of BAF, ncBAF, and PBAF complexes under these conditions?
- Which genes are affected by SWI/SNF subunit deletion, inhibition, or degradation during macrophage stimulation by RNA-seq? Do different gene classes vary in their requirement for one or more SWI/SNF complex variants?
- What are the specific regulatory relationships between transcription factors and SWI/SNF complex variants?
- How are SWI/SNF complex functions integrated? Can cooperative or antagonistic functions between SWI/SNF complex variants be identified?
- What is the contribution of SWI/SNF complexes to the formation of cell type-specific enhancers, and/or the activation of poised and latent enhancers following stimulation?
- Is the requirement for SWI/SNF subunits during stimulus-dependent responses associated with chromatin remodeling at SWI/SNF complex binding sites in cis-regulatory elements of affected genes?
- In addition to chromatin remodeling, what other functions do SWI/SNF complexes exhibit? Are any of these functions specific to a unique SWI/SNF complex variant? For example, given that BRD9 co-localizes with CTCF and Cohesin, is there a specific role for ncBAF complexes in chromatin looping or chromatin organization?
- Is the cooperative interaction between BRD9 and BRD4 observed in other cellular systems conserved in macrophages? If so, which aspects of BRD4-dependent function in inflammatory responses are BRD9-dependent? Will BRD9 bromodomain inhibitors and/or degraders be similarly beneficial in preventing sepsis and inflammation-associated diseases?
- What is the function of SWI/SNF complexes in LPS tolerance, innate immune adaptation, and trained immunity? What role do SWI/SNF complexes play in bookmarking promoters and enhancers for more rapid gene induction or repression upon restimulation?

Highlights

- SWI/SNF chromatin remodeling complexes regulate inflammatory gene expression in macrophages through interactions with lineage-determining and stimulus-regulated transcription factors.
- SWI/SNF complexes exist as three distinct variants: BAF, ncBAF and PBAF complexes. Each variant contains both shared and unique subunits, which modulate complex localization and function through subunit-specific binding interactions.
- BAF, ncBAF and PBAF complexes are enriched at different genomic elements and co-localize with distinct transcription factors.
- SWI/SNF complex variants likely regulate distinct transcriptional programs in the context of inflammatory stimulation that can be leveraged to specifically curb or promote inflammatory responses using small molecule inhibitors.
- Integration of SWI/SNF complex functions can yield greater regulatory control of complex transcriptional networks.

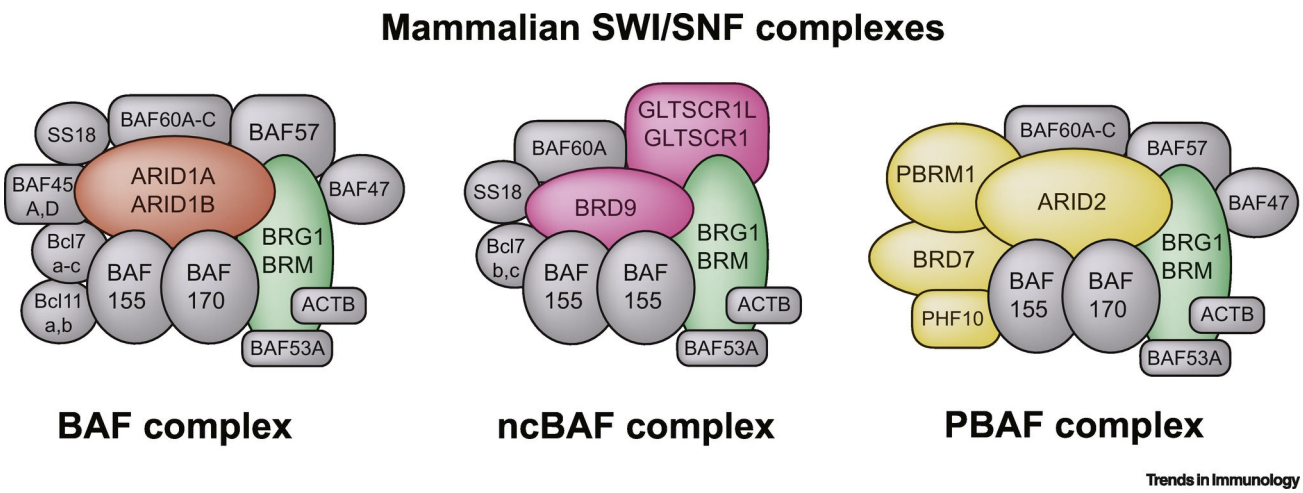

Figure 1. Biochemical heterogeneity of the mammalian SWI/SNF complexes.

Subunit composition of the three SWI/SNF complex variants -- BAF, ncBAF, and PBAF complexes -- with unique subunits colored in orange, pink, and yellow, respectively. The core ATPase subunit, either BRG1 or BRM, is colored green while other shared subunits are colored grey.

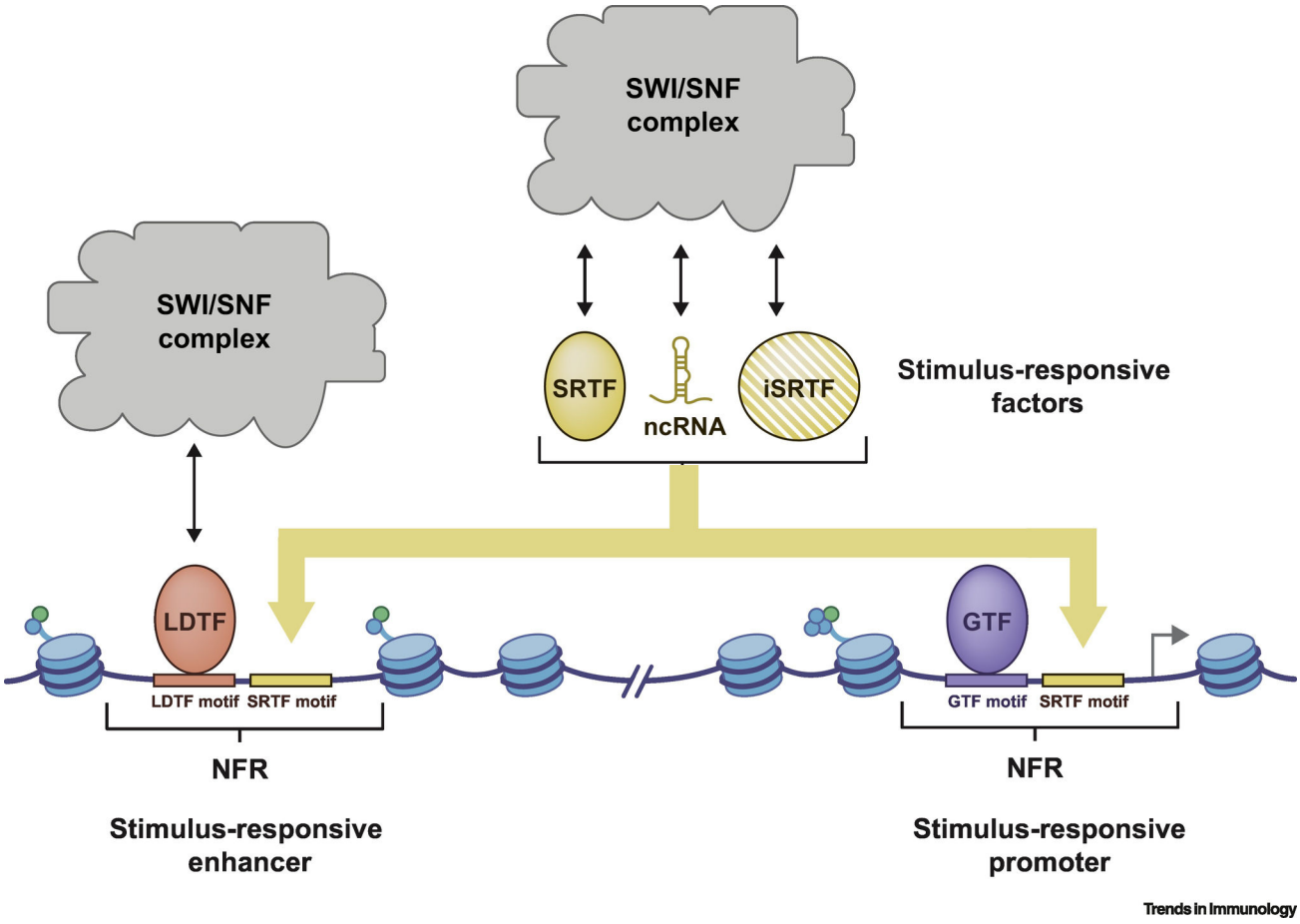

Figure 2. SWI/SNF complex recruitment to stimulus-responsive genes.

Stimulus-responsive genes are regulated by transcription factor binding to promoters and enhancers. Lineage-determining transcription factors (LDTFs) define the enhancer repertoire in a given cell type, while promoters can be pre-bound by constitutively expressed general transcription factors (GTFs). The action of LDTFs and GTFs effectively pre-marks the sites that can be bound by stimulus-regulated transcription factors (SRTFs) once cells encounter a stimulus. SWI/SNF complexes can be recruited to stimulus-responsive promoters and enhancers through interactions with LDTFs, SRTFs, non-coding RNA (ncRNA), and/or induced SRTFs (iSRTFs), where they contribute to gene induction through chromatin remodeling and regulatory interactions. Green circle: histone 3 lysine 27 acetylation; Blue circle: histone 3 lysine 4 methylation; NFR: nucleosome-free region

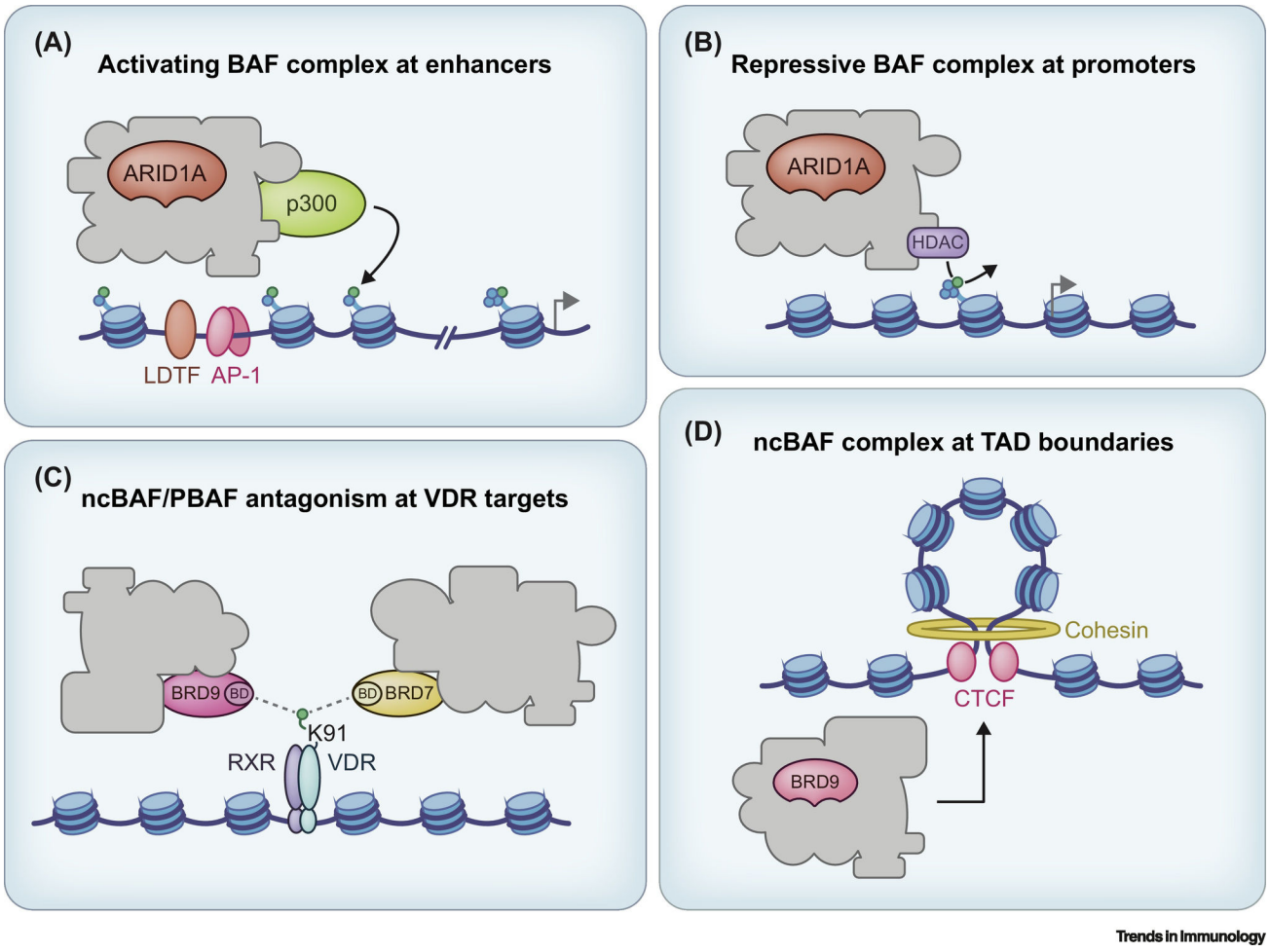

Figure 3. Localization and function of SWI/SNF complex variants.

a) ARID1A-containing BAF complexes are recruited to enhancers by LDTFs and AP-1 family members, where they are required to maintain chromatin accessibility and H3K27ac (green circles) via association with the histone acetyltransferase p300. **b)** Interaction between ARID1A-containing BAF complexes and histone deacetylases, or HDACs, at the promoters of some genes results in transcriptional repression through the removal of histone acetyl marks (green circle) by HDACs. **c)** The bromodomains (BD) of BRD9 and BRD7 have been shown to compete for binding to acetylated lysine 91 (K91) of the vitamin D receptor (VDR) (represented by the green circle on VDR), which results in antagonistic functions of ncBAF and PBAF complexes in VDR target gene expression. **d)** BRD9-containing ncBAF complexes are strongly enriched at topologically associating domain (TAD) boundaries, which are bound by the structural proteins CTCF and Cohesin.

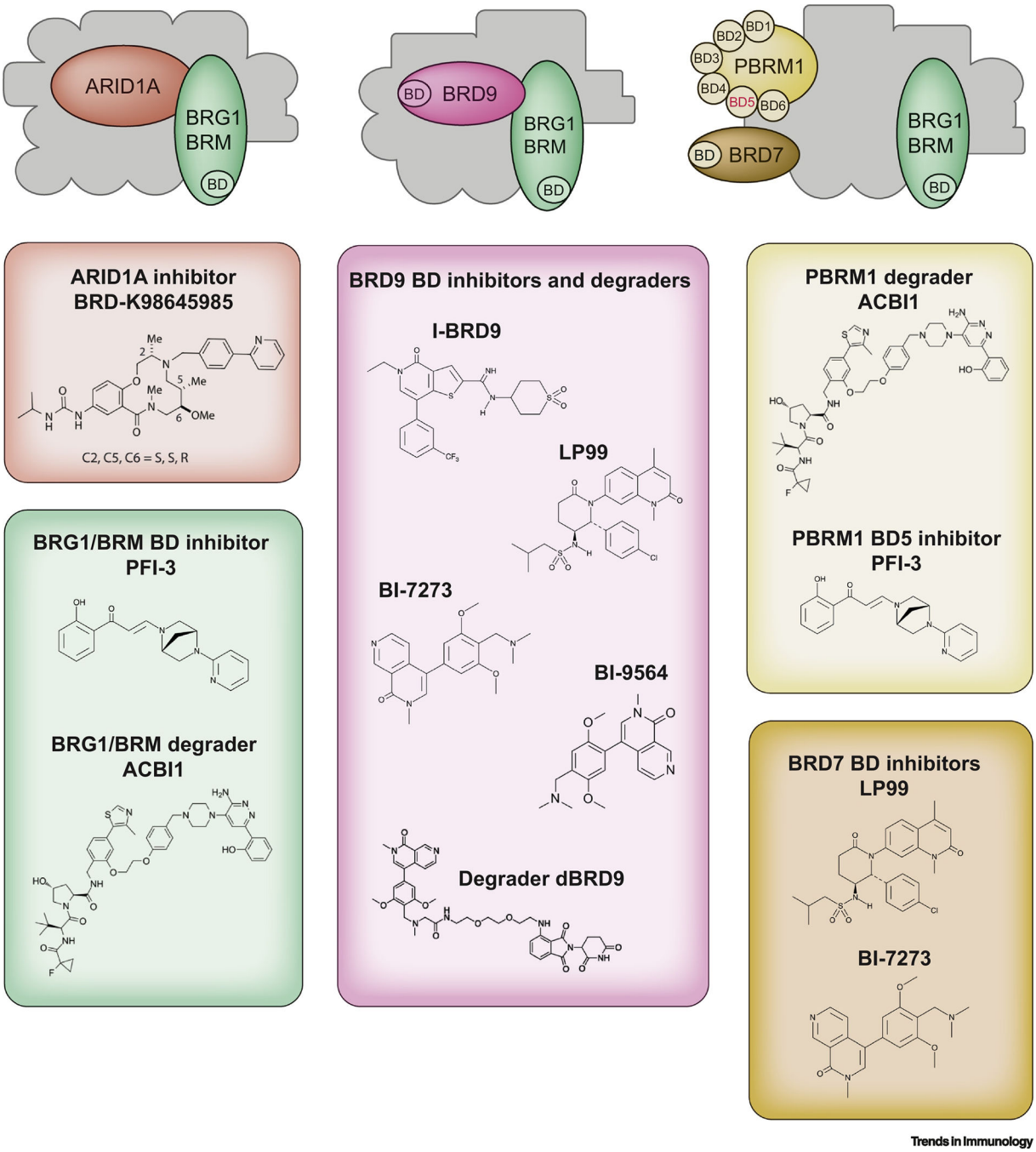

Figure 4. Small molecule inhibitors of SWI/SNF complex subunits.

Small molecules that degrade or inhibit the activities of different SWI/SNF complex subunits. All of the SWI/SNF complex inhibitors pictured here, except for the ARID1A inhibitor BRD-K98645985, are designed to competitively inhibit the binding of the bromodomain(s) (BD) within BRG1, BRM, BRD9, BRD7, and PBRM1 to acetylated lysines on histones or non-histone proteins. Note that PBRM1 harbors six BDs and that PFI-3 specifically binds to the fifth BD (red). The degraders dBRD9 and ACBI-1 are bifunctional molecules that link BD inhibitors to ligands that recruit cereblon or E3 ligase

VHL, respectively, thereby targeting the BD-containing proteins for proteasome-mediated degradation.

Table 1:

Developmental defects associated with SWI/SNF subunit deletion in murine hematopoietic lineages

Type of Deletion	SWI/SNF subunit gene	Defect	Reference
Germline	*Brg1*$^{-/-}$	Embryonic lethal	[85]
	Baf53a$^{-/-}$		[78]
	Baf45a$^{-/-}$		[79]
	Baf60b$^{-/-}$	Reduced fetal viability	[4, 5]
Hematopoietic inducible	*Baf53a*$^{f/f}$*;Mx1-Cre*	Reduced BM cellularity; impaired HSC and progenitor proliferation	[78]
	Baf45a$^{f/f}$*;Mx1-Cre*	Reduction of LT-HSC and myeloid progenitors	[79]
	Baf60b$^{f/f}$*;Mx1-Cre*	Impaired development of granulocytes and eosinophils	[4, 5]
	Arid1a$^{f/f}$*;Mx1-Cre*	Loss of quiescence in HSC; disrupted differentiation of both myeloid and lymphoid lineages	[2]
	Baf155$^{f/f}$*;Mx1-Cre*	Defects in common lymphoid progenitors; block in B cell development at the transition from pre-pro-B to early pro-B cells	[86]
B cell specific	*Brg1*$^{f/f}$*;IL7r-Cre*	Block in B cell development at the pro-B cell stage; *Igh* locus decontraction, disruption of Vh-DhJh recombination	[12]
	Baf155$^{f/f}$*;CD19-Cre*	Loss of germinal center B cell and follicle helper T cell expansion after immune challenge	[87]
T cell specific	*Lck-Baf57ΔN*	Impaired CD4 silencing (enhanced by heterozygous brg1 null allele) and CD8 expression	[88]
	Brg1$^{f/f}$*;Lck-Cre*	Impaired CD4 silencing; block in thymocyte development at DN3	[89, 90]
	Baf47$^{f/f}$*;Lck-Cre*	Block in thymocyte development at DN3; development of peripheral T cell lymphomas	[91]
	Baf47$^{f/f}$*;CD4-Cre*	Normal thymocyte development; development of peripheral T cell lymphomas	[91]

Table 2:

Effects of SWI/SNF complex inhibitors

Inhibitor	Effect	Reference
BRD-K98645985	HIV-1 latency reversal in primary human T cells	[70]
PFI-3	No anti-proliferative effect on human cancer cell lines; enhanced differentiation of mouse trophoblast stem cells	[92, 93]
ACBI1	Induced cell death in BRG1-deficient human cancer cell lines	[69]
I-BRD9	Loss of naïve pluripotency in mouse embryonic stem cells; restored function in the rat β cell INS-1 cell line and improved glucose homeostasis in a mouse type 2 diabetes *db/db* model; reduced viability in SYO-1, HSSYII human synovial sarcoma cell lines	[14, 56, 57]
LP99	Reduced LPS-induced IL-6 expression in human monocyte THP1 cell line	[94]
BI-7273	Reduced cell viability in SYO-1, HSSYII human synovial sarcoma cell line	[56]
BI-9564	Loss of naïve pluripotency in mouse embryonic stem cells	[14]
dBRD9	Anti-proliferative effects in SYO-1, HSSYII human synovial sarcoma and TTC1240 human malignant rhabdoid tumor cell lines; blocks *in vivo* synovial sarcoma tumor progression in xenografts	[15, 56]