

THE BROMODOMAIN: A REGULATOR OF ATP-DEPENDENT CHROMATIN REMODELING?

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. The ATP-dependent chromatin remodeling family
4. The bromodomain: A complex regulatory domain?
 - 4.1. Bromodomain function in ATP-dependent chromatin remodeling complexes
 - 4.2. Potential models for bromodomain function
 - 4.2.1. Bromodomains as direct targeting domains
 - 4.2.2. Bromodomains as mediators of complex exchange following recruitment
 - 4.2.3. Bromodomains as allosteric regulators of remodeling complex function
5. Acknowledgements
6. References

1. ABSTRACT

In eukaryotes, processes requiring access to DNA are inhibited by the structural packaging of the genome. A number of specialized ATP-dependent chromatin remodeling enzymes have evolved to overcome this inhibition. One subset of these enzymes, SWI/SNF, plays a critical role in the regulation of transcription, often functioning in concert with nuclear histone acetyltransferases (HATs). It remains unknown how these activities are coordinated. However, recent results revealing that the bromodomain, a motif common in these remodeling factors, constitutes an acetyl-lysine binding domain might provide insight into this process. Bromodomains may serve a role analogous to the signal transduction SH2 domain, by providing a means to recruit remodeling complexes to acetylated chromatin regions or to allosterically modify their function post-recruitment.

2. INTRODUCTION

The DNA of eukaryotes is encompassed in a hierarchical structure of 30-400 nm chromatin fibers composed of histone and non-histone proteins (for review see (1)). Two major classes of eukaryotic enzymes have evolved to overcome the obvious obstacles presented by such a complex structure to any process requiring access to DNA. The first family is composed of an increasingly diversifying group of ATP-dependent chromatin

remodeling enzymes that utilize the energy of ATP hydrolysis to facilitate factor access to chromatin sites (2-4). The second class, composed of a family of histone acetyltransferases (HATs), acetylates critical lysine residues within the N-terminal "tails" of the core histones (5, 6). In several cases, these two classes of enzymes are both simultaneously involved in the regulation of specific genes (7, 8). However, it remains unclear how their function is coordinated. Recent identification of the bromodomain as an acetyl-lysine binding domain (9-11) suggests that this domain might play a role in complex cooperation by serving as a targeting domain, or alternatively by serving as a sensor of acetylation and HAT function.

3. THE ATP-DEPENDENT CHROMATIN REMODELING FAMILY

Each member of the ATP-dependent chromatin remodeling family consists of a multisubunit complex dependent upon a single ATPase subunit with sequence homology to the DEAD/H family of helicases (12). The ATPase subunits share significant homology within a central core domain constituting the ATPase domain, but display considerable divergence within their N-terminal and C-terminal sequences. The ATP-dependent complexes can be further subdivided into three distinct classes based

The Bromodomain and Chromatin Remodeling

upon their degree of similarity to one of three prototype ATPases: the SWI/SNF subclass (defined by greatest homology to the yeast SWI2/SNF2 ATPase), the ISWI subclass (defined by similarity to the *Drosophila* ATPase ISWI), and the Mi-2 subclass (defined by similarity to the CHD ATPases) (2). Sequences C-terminal to the ATPase domain are also conserved within each individual subclass, with each subclass carrying a characteristic C-terminal signature domain: bromodomains are present in SWI/SNF related complexes, SANT domains in ISWI complexes, and chromodomains within CHD/Mi-2 family members.

While the precise mechanistic function of these domains remains unknown, a role in complex regulation seems most likely. The bromodomain has been shown to bind acetylated lysine residues within the N-terminal histone tails (9-11) and recent results suggest that the chromodomain could serve an analogous function in recognition of methylated lysine residues (13, 14). Conceivably, these domains could play a regulatory role by monitoring modifications of the chromatin substrate—perhaps allowing these remodeling enzymes to sense the presence of their common partners, acetyltransferases and methyltransferases.

4. THE BROMODOMAIN: A COMPLEX REGULATORY DOMAIN?

The bromodomain, an approximately 100 amino acid module ubiquitously found in eukaryotes, was originally identified as a conserved sequence element of unknown function in a handful of genes including the *Drosophila* *Brm* and *fsh* genes, the *S. cerevisiae* SWI2/SNF2 and SPT7 genes, and two human genes, CCG1 and RING3, many of which can be implicated in gene regulation (15, 16). Jeanmougin and coworkers expanded both the family and the motif, identifying 37 different members including a number of additional HATs, as well as several SWI2/SNF2, *fsh* and RING3 homologues (17). The fact that many of these proteins participate directly in gene regulation through chromatin modification suggests a potential mechanistic role for the domain, however the domain is also found in several proteins not directly participating in chromatin modification—the *Drosophila* *fsh* gene and the related RING3 gene encode nuclear protein kinases (18), and several of the non-enzymatic components of the ATP-dependent remodelers (e.g. RSC1/2/3 and Polybromo) also contain essential bromodomains (19). Overall, bromodomain-containing proteins have been implicated in functions as diverse as cell-cycle control (CCG1 and RING3) (18), cellular differentiation (the *Drosophila* genes *fsh* and *brm*) (20), transcriptional regulation (SWI/SNF and RSC), and mitotic chromatin dynamics (21).

The solution structures of the HAT bromodomains provide an important hint to bromodomain function (9-11). The domain consists of an antiparallel left-handed four helix bundle with two long loops between the first and second and third and fourth helices that define an apparent acetyl-lysine binding site. Acetyl-lysine recognition occurs through residues in the ZA loop

(residing between helices 1 and 2) and BC loop (between helices 3 and 4). The fact that these amino acid residues are well conserved in all family members argues that this binding activity is a general feature of bromodomains (9). Interestingly, the observation that the chromodomain binds methylated lysine provides a structural parallel between the SWI/SNF and CHD family of complexes (13, 14). This similarity suggests a model in which either the bromodomains or chromodomains of these respective ATPases could function to regulate complex function. An interesting prediction of this hypothesis is that the SANT domain of the remaining subclass might fulfill a similar role in ISWI complex function.

4.1. Bromodomain function in ATP-dependent chromatin remodeling complexes

While only a limited amount of data is available concerning bromodomain function in the ATP-dependent remodeling complexes, the identification of the domain as an acetyl-lysine binding domain suggests that a closer look into its role may be warranted. In yeast, a partial deletion of the C-terminus of yeast SWI2 has no apparent effect on SWI2/SNF2 function in vivo (22, 23). This SWI2/SNF2 mutant is still capable of supporting wild-type growth on sucrose despite a complete disruption of the bromodomain, although it remains possible that proper functioning of the complex at other loci could require an intact bromodomain. This is particularly true in the case of more complex promoters, such as the *HO* gene promoter, which require very precise coordination of SWI/SNF and HAT complex activities (7, 8). The effect of such a bromodomain deletion on *HO* promoter function remains unknown. However, it is known that deletion of bromodomain sequences from the *Drosophila* *Brm* gene also does not disrupt zygotic or maternal gene functions (24). The lack of any apparent phenotype mirrors the behavior of the SWI2/SNF2 bromodomain deletion in yeast, and suggests that the bromodomain may be dispensable for the function of SWI/SNF-related complexes.

The SWI/SNF family contains a second closely related complex, yeast RSC. The two complexes, although closely related, play distinct roles in vivo. While SWI/SNF is required for the transcription of a subset of yeast genes, the RSC complex appears to play a more global role in cell growth (25). Interestingly, the RSC complex contains multiple bromodomains critical for complex function within both the ATPase and accessory subunits. Deletion of the STH1 gene encoding the RSC ATPase subunit is lethal for vegetative growth, with mutant yeast undergoing cell-cycle arrest at the G2/M boundary. While an STH1 bromodomain deletion derivative is viable, this mutant displays a temperature-sensitive cell-cycle block (26) similar to that due to mutations in other RSC complex components (27), implying a role for the domain in coordinating complex function. Two additional RSC subunits, RSC1 and RSC2, also contain essential bromodomains (19). These two highly related proteins, apparently present in distinct RSC complexes, each contain two tandem bromodomains, only one of which is essential to complex function (28). The mammalian RSC counterpart, SWI/SNF-B (aka PBAF), contains a related

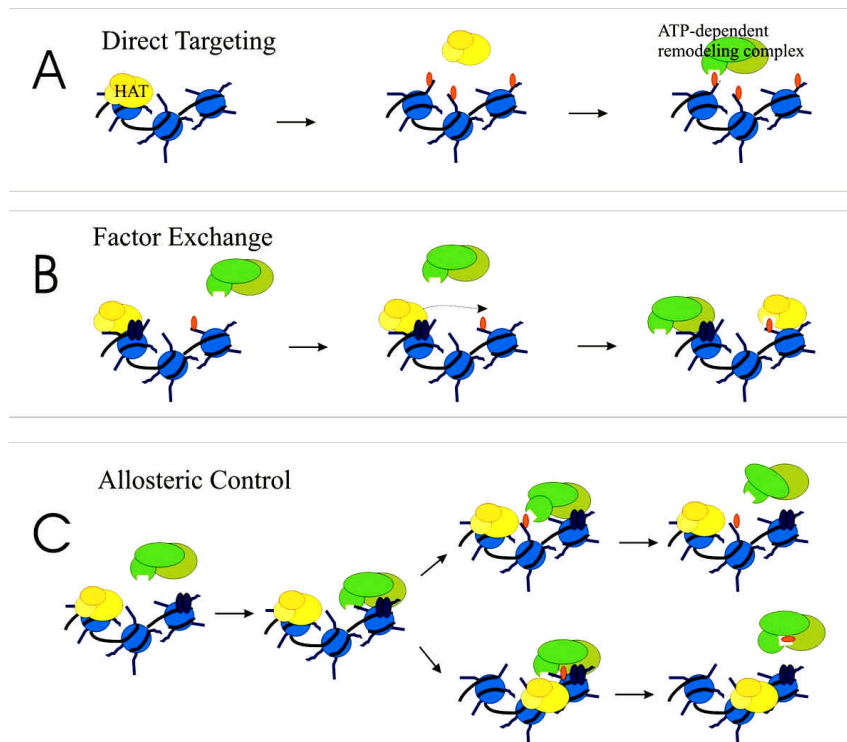


Figure 1. Three potential models for bromodomain function in ATP-dependent remodeling. A segment of chromatin is depicted in blue with the histone tails extended. An ATP-dependent remodeling complex is shown in green, with the bromodomain represented by the notched subunit. A HAT complex is depicted in yellow, with acetylated lysine residues represented by the red ovals. Panel A. Bromodomains might function directly to recruit ATP-dependent chromatin remodeling complexes to chromatin pre-acetylated by HAT complexes. Panel B. Bromodomains might facilitate the exchange of complexes post-recruitment. Panel C. Bromodomains might serve as an allosteric sensor of chromatin acetylation triggering dissociation of the complex (top pathway) or alternatively as a sensor of acetylation of the remodeling complex itself (bottom pathway). In this schematic, acetylation of the ATP-dependent remodeling complex by a neighboring HAT complex triggers a conformational change in the ATP-dependent remodeling complex masking the active site.

subunit BAF180 with 5 tandem bromodomains. The protein appears to have evolved by fusion of the RSC1, RSC2 and RSC4 genes. Unfortunately, no mutagenesis has been performed on this subunit, however such a protein could be envisioned to play a role in targeting of the mammalian RSC complex to specifically hyperacetylated chromatin regions.

4.2. Potential models for bromodomain function

How could the bromodomain play a role in ATP-dependent chromatin remodeling? The acetyl-lysine specificity is suggestive of a role analogous to SH2 domains in protein kinase mediated signal transduction, with the bromodomain serving either as an adaptor to allow recruitment to specifically modified protein partners, or as an allosteric effector of complex activity in response to acetylation of a partner or substrate (Figure 1). Participation could occur at several points in complex function: (1) bromodomains might directly target complexes to pre-acetylated chromatin, (2) provide a mechanism for transcriptional activators to release complexes in preparation for recruitment of another complex, or (3) modulate complex ATPase activity allosterically by sensing the presence or absence of histone acetylation.

4.2.1 Bromodomains as direct targeting domains.

Under such a model, complex recruitment would be mediated directly by histone acetylation, with remodeling complexes following HAT complexes to properly tagged chromatin (Figure 1A). While this model is attractive due to its simplicity, the relatively high K_d (~100 micromolar) for the bromodomain acetyl-lysine interaction argues against this model (9), since it is difficult to reconcile how an interaction with such low affinity might be used to directly target complexes. However, in the case of proteins containing tandem bromodomain repeats, (e.g. TAF_{II}250 or the Polybromo subunit of hSWI/SNF-B), multimerization of the domain may sufficiently enhance affinity to allow strong recognition of specifically modified nucleosomal sites. This is observed in the case of TAF_{II}250, a subunit of the transcription factor TFIID. In this case, two tandem bromodomains enhance the protein's affinity for its probable target, a specifically diacetylated histone tail (11).

4.2.2. Bromodomains as mediators of complex exchange following recruitment

While the bromodomain's affinity for acetyl-lysine may be too modest to allow for direct complex

recruitment, that does not exclude the possibility that bromodomains could provide a secondary means to tether complexes post-recruitment. The fact that many transcriptional activators are capable of recruiting both SWI/SNF and SAGA complexes to chromatin produces somewhat of a conundrum (for examples see (29)). How do these activators choose between partners to recruit, and how do these activators coordinate the entry and exit of individual complexes when recruitment of multiple complexes is necessary? Control of the order of recruitment may result simply from differential affinity of activator for individual complexes—higher affinity complexes arrive first or leave slowest. Redundancy of transcription factor binding sites could then provide the opportunity for the same activator to recruit multiple complexes, with simple probability governing which complex arrives first and how long each remains associated. Alternatively, complex recruitment may occur in an ordered fashion, with bromodomains allowing dissociation from the activator followed by reassociation with acetylated chromatin (Figure 1B). This target and release model would provide a means for one activator to recruit a complex, and then release that complex to an adjacent region of the promoter via the inherent “stickiness” of a localized region of acetylated chromatin. Following release, the same activator would then be free to recruit a second complex to proceed with the process of promoter activation.

4.2.3. Bromodomains as allosteric regulators of remodeling complex function

The previous models involve direct participation of the bromodomain in mediating complex affinity. Alternative models with the domain playing an indirect role are equally plausible. Conceptually, the domain could behave primarily as a sensor of chromatin acetylation state and/or a sensor of the presence of additional remodeling complexes. In this model, acetylation of chromatin could signal conformational rearrangements that alter ATPase activity or chromatin substrate recognition, triggering an end to ATP-dependent remodeling, or inducing spreading to adjacent chromatin and/or dissociation of the complex from the site (Figure 1C). Intriguingly, a number of transcription factors are also targets of acetyltransferases (30), raising the potential regulatory complexity of the system—any transcription factor acetylation event could also trigger allosteric changes in remodeling enzyme activity, association or dissociation from the activator. From this perspective, bromodomains, chromodomains, and SANT domains may prove to be the SH2 domains of the nuclear signal transduction pathway.

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