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Structural features and inhibitors of bromodomains

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Bromodomains are conserved structural modules responsible for recognizing acetylated-lysine residues on histone tails and other transcription-associated proteins, such as transcription factors and co-factors. Owing to their important functions in the regulation of ordered gene transcription in chromatin, bromodomains of the BET family proteins have recently been shown as druggable targets for a wide array of human diseases, including cancer and inflammation. Here we review the structural and functional features of the bromodomains and their small-molecule inhibitors. Additional new insights provided herein highlight the landscape of the ligand binding sites in the bromodomains that will hopefully facilitate further development of new inhibitors with optimal affinity and selectivity.

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Introduction

The great diversity of the human proteome is due in part to the power of post-translational modifications (PTMs) [1], which are small chemical groups added to amino acid residues in proteins. These modifications have the ability to affect the functions of proteins, their cellular localization and the manner in which they interact with other proteins. Specifically, the post-translational modification of histones, the proteins around which DNA is wrapped to form nucleosomes, plays an essential role in the regulation of gene transcription in chromatin. Acetylation of lysine residues on histone tails is among the most abundant PTMs, and the protein domains responsible for recognizing these modifications are called bromodomains (BrDs). These functional domains are primarily responsible for reading these acetyl marks within the cell, but some have also recently shown to be capable of recognizing other PTMs, such as lysine butyrylation and lysine crotonylation [2]. As the reader of these marks, the BrDs plays a central role in the complex biological system of gene transcription in chromatin, as BrD-containing proteins are pivotal in recruiting chromatin remodeling proteins and different transcription factors to active transcription sites. Improper recognition of lysine PTMs in gene transcription has been linked to numerous human diseases including cancer, which brings BrD-containing proteins to the forefront as new therapeutic targets. This review highlights structural and functional features of BrDs, their chemical inhibitors as well as current drug candidates being evaluated in human clinical trials.

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Structural coverage of the bromodomains

A total of 42 genes encodes for at least 56 bromodomains in the human genome [3], and each of the BrD-containing protein contains between one and six BrDs. The first three-dimensional structure of a BrD (specifically, that of the histone acetyltransferase KAT2B also known as PCAF) was solved in 1999, using NMR spectroscopy [4]. This study also demonstrated for the first time the BrD function as a conserved acetyl-lysine (Kac) binding domain for histones. The structure was the first of many to exhibit the unique BrD fold as four alpha helices with a left-handed fold, denoted as α_Z, α_A, α_B, α_C (Fig. 1a). In addition, the unique BrD structure contains two loops one connecting the α_Z and α_A helices (called the ZA loop), and a second connecting the α_B and α_C helices (the BC loop). This NMR study used 2D ^{1}H–^{15}N heteronuclear single quantum coherence (HSQC) spectra to establish that a lysine-acetylated peptide perturbed residues between the ZA and BC loops defined as the Kac binding site in BrDs (Fig. 1b), providing direct biochemical evidence that acetylated lysine binds at this location. One of the interesting features in the Kac binding site that was later confirmed is the presence of four conserved and difficult to displace water molecules, one of which is in direct contact with the acetylated lysine tail (Fig. 1c). It is important to note that the BrDs are often found to be associated with other epigenome reader domains within the context of a given BrD-containing protein [5] (e.g. plant homeodomains (PHDs), Pro-Trp-Trp-Pro (PWWP) domains) or other enzymatic domains (such as histone acetyltransferases (HATs)). These associations highlight the contribution of BrDs to the complex combinatorial recognition process in the regulation of gene transcription in chromatin [6,7].

BrDs can be classified into 8 subgroups [8] according to the similarity of their sequence (Fig. 2a). A total of 453 structures of 41 BrDs have been solved to date [9] (June, 30th 2016) with at least one crystal structure available for each subgroup. Due to massive interest in the biology and the development of chemical inhibitors for some BrDs, certain subgroups have been more investigated than others. Group II contains BET (bromodomain and extra-terminal family) BrDs (BRD2, BRD3, BRD4 and BRDT) and has the highest number of crystal

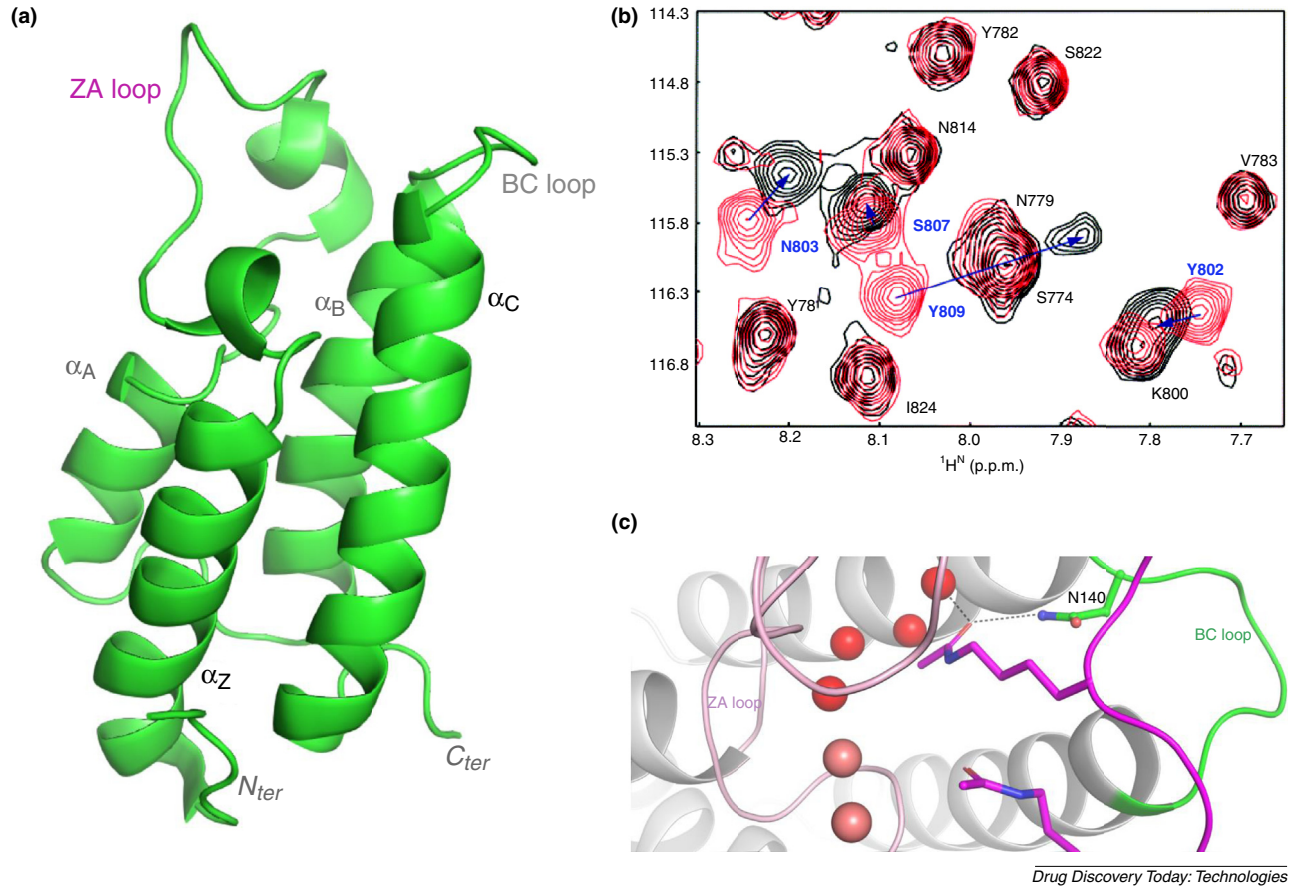

Figure 1. Structure of the BrD fold and function. **(a)** The 3D structure of the conserved BrD fold. Highlighted are the four helices (α_Z, α_A, α_B, α_C), the ZA and BC loops that connect them, and the N and C termini of the bromodomain. **(b)** Superimposed region of the 2D ^{15}N-HSQC spectra of the BrD (0.5 mM) in its free form (red) and in complex with the Kac-containing H4 peptide (black) (molar ratio 1:6), adapted from Dhalluin *et al.* [4]. **(c)** The Kac binding site of a BrD, illustrated by a structure of BRD4-BrD1 in complex with H4K5acK8ac (pdb: 3UVW), with the four conserved water molecules, present in most of the BrDs, colored in red. Other water molecules in this specific binding pocket are colored in pink.