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Structure of a BCOR corepressor peptide in complex with the BCL6 BTB domain dimer

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SUMMARY

The transcriptional corepressors BCOR, SMRT and NCoR are known to bind competitively to the BCL6 BTB domain despite the fact that BCOR has no detectible sequence similarity to the other two corepressors. We have identified a previously uncharacterized 17 residue motif from BCOR that binds directly to the BCL6 BTB domain and determined the crystal structure of the complex to a resolution of 2.6 Å. Remarkably, the BCOR BCL6 Binding Domain ($BCOR^{BBD}$) peptide binds in the same BCL6 binding site as the $SMRT^{BBD}$ peptide despite the lack of any significant sequence similarity between the two peptides. Mutations of critical $BCOR^{BBD}$ residues cause the disruption of the BCL6 corepression activities of BCOR, and a $BCOR^{BBD}$ peptide blocks BCL6-mediated transcriptional repression and kills lymphoma cells.

INTRODUCTION

BCL6 is a potent transcriptional repressor with critical roles in several immunological processes including germinal center development, generation and maintenance of memory T cells, and chemokine gene expression in T helper cells and macrophages (recently reviewed in Jardin et al., 2007). BCL6 is highly expressed in B-cells undergoing affinity maturation within germinal centers, and its expression is downregulated upon selection for apoptosis or differentiation (Cattoretti et al., 2006). In addition to its normal roles, BCL6 is associated with diffuse large B-cell lymphoma (DLBCL) and follicular lymphomas (FL), where deregulated expression of the protein due to chromosomal translocations and somatic hypermutations in the autoregulatory region of the gene can detain cells in the GC stage by blocking plasmacytic differentiation and apoptosis (Jardin et al., 2007). BCL6 has been shown to play a central role

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in the pathogenesis of DLBCL (Cattoretti et al., 2005), and primary DLBCLs with increased BCL6 expression have a characteristic transcriptional signature (Polo et al., 2007). Inhibitors of BCL6 repression show promise as therapeutic agents for B-cell lymphomas (Polo et al., 2004).

BCL6 is a 706 amino acid protein consisting of an N-terminal BTB/POZ domain, followed by a central region of approximately 400 residues that is predicted to have little or no fixed structure, and terminated by six C-terminal C_2H_2-type zinc finger (ZF) DNA binding motifs. The BTB domain is a highly conserved protein-protein interaction motif present in over 200 human BTB proteins, forty-three of which are BTB-ZF proteins with a similar architecture as the BCL6 protein (Stogios et al., 2005). In all cases known to date, BTB domain dimerization, and possibly BTB-mediated oligomerization, is required for the normal activities of the BTB-ZF proteins.

The BCL6 repression activity involves the recruitment of class I and class II histone deacetylase complexes (HDAC) to BCL6 recognition sites through both direct contacts with HDAC complexes (Lemercier et al., 2002) and indirect contacts bridged by corepressors (Lemercier et al., 2002; Wong and Privalsky, 1998). Corepressors that are known to interact with BCL6 include BCOR (BCL6 interacting corepressor) (Huynh et al., 2000), SMRT (also known as NCoR2) (Dhordain et al., 1997; Huynh and Bardwell, 1998; Wong and Privalsky, 1998) and NCoR (Huynh and Bardwell, 1998). These corepressors bind in a mutually exclusive fashion to the BTB domain of BCL6, suggestive of overlapping binding sites (Huynh et al., 2000). SMRT and NCoR use a nearly identical 17 residue BCL6 binding domain (BBD) to associate with BCL6, and we previously determined the crystal structure of the BCL6 BTB domain in complex with the SMRT BBD ($SMRT^{BBD}$) (Ahmad et al., 2003). A pTAT verision of the $SMRT^{BBD}$ peptide is able to inhibit BCL6 corepressor recruitment and leads to apoptosis and cell cycle arrest of BCL6 positive lymphoma cell lines (Polo et al., 2004).

BCOR was originally identified as a novel BCL6 corepressor (Huynh et al., 2000). It is strongly and specifically expressed during embryonic development (Wamstad and Bardwell, 2007) where it plays a critical role in transcriptional regulation, as BCOR mutations are associated with the X-linked inherited diseases Lenz microphthalmia and Oculofaciocardiodental syndrome (Ng et al., 2004). BCOR copurifies with Polycomb group (PcG) transcriptional repressor proteins and Skp-Cullin-F-box (SCF) ubiquitin E3 ligase components (Gearhart et al., 2006; Sanchez et al., 2007), and the complex can monoubiquinate histone H2A (Gearhart et al., 2006). All known BCOR isoforms are able to bind to the BCL6 BTB domain, but there are no identifiable motifs in BCOR that have sequence similarity to the SMRT/NCoR BBD.

In this study we identify the minimal BCOR BBD ($BCOR^{BBD}$) and determine its crystallographic structure in complex with the BCL6 BTB domain ($BCL6^{BTB}$). We show that despite no detectable sequence similarity between the BCOR and SMRT minimal peptides, both bind to very similar surfaces of the $BCL6^{BTB}$ dimer. There are important differences in many of the specific interactions, and the BCOR and SMRT peptides have different determinants for recognition of the same $BCL6^{BTB}$ binding site. Finally, we validate the $BCL6^{BTB}/BCOR^{BBD}$ interaction and show that the $BCOR^{BBD}$ peptide inhibits BCL6 corepressor recruitment in an analogous fashion to the $SMRT^{BBD}$ peptide.

RESULTS

BCOR residues 498−514 comprise the minimal BCL6 Binding Domain

BCOR was first identified in a yeast two-hybrid screen to detect proteins that bind to the N-terminal half of BCL6 (Huynh et al., 2000). The original clone was a partial cDNA consisting of BCOR residues 112−753, and subsequent cloning and sequence analysis of the full-length

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gene revealed that BCOR does not share significant similarity with any other human protein, with the exception of a motif of three consecutive ankyrin motifs from residues 1428−1527 (Huynh et al., 2000). More recent functional studies have identified an AF9 binding site from residues 1157−1251 (Srinivasan et al., 2003) and an NSPC1 binding site within residues 1428 − 1721 (Gearhart et al., 2006). With the exception of the ankyrin region, BCOR is predicted to be mostly unstructured in solution.

In order to determine the minimal BCOR BCL6 binding domain, we expressed and purified a series of overlapping BCOR fragments as histidine-tagged thioredoxin fusion proteins, and analyzed their ability to bind to purified $BCL6^{BTB}$ on Ni-NTA affinity resins. $BCL6^{BTB}$ co-purified with BCOR residues 317−547, but not with residues 112−342 or 542−753 (data not shown). Subfragments of the 317−547 region were then assayed for association with $BCL6^{BTB}$ by a native gel electrophoresis assay, leading to the identification of the region from 498 to 514 as the minimal binding region (Figure 1A,B). Deletion of residues from either the N-terminus or C-terminus of BCOR 498−514 resulted in a loss of binding to $BCL6^{BTB}$. The dissociation constant (K_d) between $BCL6^{BTB}$ and $BCOR^{498-514}$ was 1.32 +/− 0.02 μM (average of three titrations) by isothermal titration calorimetry (Figure S1 in the Supplementary Data) with a stoichiometry of interaction of 1.13 +/− 0.01. Because the BCL6 domain is an obligate homodimer, the corepressor complex exists as a 2:2 complex in solution. There is no indication of cooperativity in the ITC data.

Structure of the $BCL6^{BTB}$/$BCOR^{BBD}$ complex

We reported previously that the $SMRT^{BBD}$ binds in a lateral groove that is found at the interface of the two chains of the $BCL6^{BTB}$ dimer (Ahmad et al., 2003). The binding of SMRT and BCOR to BCL6 is mutually exclusive (Huynh et al., 2000), suggesting that $BCOR^{BBD}$ might interact with a similar region of the BTB lateral groove. In order to determine the nature of the BCL6/BCOR binding interface, we co-crystallized $BCL6^{BTB}$ with $BCOR^{BBD}$, and solved the structure to a resolution of 2.6 Å (Figure S2 and Table S1).

There are a total of four independent BTB dimers within the crystal asymmetric unit, and each dimer is associated with two $BCOR^{BBD}$ peptides (Figure 1C). Thus, the crystals provided 8 independent views of the protein-peptide interface. A superposition of the structures revealed little difference between individual protein and peptide chains (Figures S3 and S4), with pairwise Cα rmsd values ranging from 0.38 to 0.60 Å.

The BCOR BBD associates with the same region of $BCL6^{BTB}$ as the SMRT BBD and follows a similar path along the BTB dimer surface. As in the SMRT complex (Ahmad et al., 2003), the six N-terminal residues of $BCOR^{BBD}$ contribute a third strand to the lower β-sheet of the BCL6 dimer made up from BTB strands β1 and β5. The rest of the BBD peptide then winds up along the lateral groove at the interface of the BTB dimer, with each peptide forming contacts to both BCL6 chains. Since the peptide binding site is at the interface between the two chains, the interaction is only possible in a BTB dimer.

The $BCL6^{BTB}$ main chain is not significantly affected by the binding of $SMRT^{BBD}$ or $BCOR^{BBD}$, relative to the unliganded state. However, BCL6 side chains R13, R24 and H116 adopt significantly different conformations in the BBD complexes (Figure S5). In both corepressor complexes, a repositioning of BCL6 residue H116 is required to prevent steric clashes with the BBDs, but unlike residues R13 and R24, the conformation of H116 in liganded state is corepressor specific (see below).

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Comparison of the BCOR^{BBD} and SMRT^{BBD} complexes

The BCOR and SMRT peptides share some regions of common interactions, but some regions have distinctly different interactions (Figure 2). The BBDs adopt significantly different structures in the center of the peptides, resulting in very different backbone conformation and BTB-BBD interactions over BCOR residues A505 - S508 and SMRT residues G1422 - I1425 (Figure 2). The BCOR and SMRT peptides are structurally well defined in their respective complex structures, as evidenced by the small error bars in Figure 2E.

The BCL6 H116 conformation observed in the SMRT^{BBD} complex is not sterically possible when BCOR^{BBD} is bound and vice versa (Figures 2A, 2C, S5 and S6). The change in the H116 rotamer produces significantly different surfaces on the BCL6 lateral groove, and the BBD peptides accordingly make different corepressor-specific interactions in this region. For example, S1424 and I1425 of SMRT are buried deep in the lateral binding groove and are covered by residue H116 of BCL6. In contrast, BCOR residues S507 and S508 are exposed to solvent because H116 rotates into the binding groove and packs beneath the peptide. The hydrophobic SMRT residue I1425 is a key "anchor" residue that sits in a deep apolar pocket in the BTB lateral groove, but the corresponding BCOR residue S508 is flipped out and forms a solvent-exposed side-chain hydrogen bond to the backbone amide of BCL6 residue E115.

The structure-based alignment of the two BBDs peptides reveals strikingly few conserved residues (Figure 2E), such that no real "binding motif" can be described at the sequence level. Note that the serine residues BCOR S507 and SMRT S1424 form entirely different main chain and side chain contacts in their respective complexes, and are thus not homologous (Figures 2 and 3). This leaves the C-terminal proline (BCOR P512 / SMRT P1429) as the only strictly conserved residue between the two peptides, but this residue contributes only a single main-chain hydrogen bond to the side chain of BCL6 residue R28.

An analysis of the interactions made by the peptides reveals a combination of conserved backbone interactions and a few key corepressor specific interactions. The majority of the interactions that are conserved between SMRT and BCOR complexes are polar in nature and mostly involve the main chain atoms over the entire length of the BBDs (Figure 3A). In contrast, the majority of the non-conserved BCL6 BTB interactions are apolar and involve the side chains from the C-terminal half of the BBDs (Figure 3B). In the case of SMRT, residues R1423, S1424 and I1425 make the most important SMRT-specific interactions. The long hydrophobic side chains of R1423 and I1425 form significant VDW contacts with BCL6, while S1424 forms unique polar and non-polar interactions. In the case of BCOR, the large W509 side chain forms an extensive network of contacts with several BCL6 side chains. The analogous H1426 in SMRT makes many fewer BCL6 contacts, however, it is remarkable that similar contacts are made by the imidazole rings of BCOR W509 and SMRT H1426 (Figure S6).

Importance of individual BBD side chains in BCL6 binding

We tested a series of SMRT^{BBD} and BCOR^{BBD} peptides with single residue substitutions for BCL6^{BTB} binding with a fluorescence polarization (FP) assay (Figures 4, S7 and S8). Amino acids were replaced by alanine, except in positions where alanine was the natural amino acid, in which case glycine was used. In general, positions of the N-terminal half of both the BBDs tolerated most substitutions, while the side chains in the C-terminal halves of the peptides were more important to complex formation. There is a good general correlation between the extent of side chain burial and the importance for binding (Figures 3 and 4C). In particular, substitutions at BCOR S508 and SMRT I1425 resulted in a large loss of affinity, despite the fact that these residues make very different contacts to the BCL6 BTB domain in the respective complexes (Figures 2 and 3).

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Mutation of the BCOR^{BBD} specifically affects its ability to function with BCL6

The mapping and structural studies provided a high-resolution view of the interaction of BCOR with BCL6. Next we wanted to determine whether this interaction is essential for the BCOR corepression activity with BCL6. A triple BCOR mutant (S507A/W509A/V511A) was tested in a cotransfection coimmunoprecipitation assay with full-length proteins. A triple mutant was chosen instead of a single mutant since, in previous studies, mutations that reduced SMRT^{BBD} interactions in biochemical assays to below measurable levels retained weak residual binding activity in biological assays (Polo et al., 2004). Both wild type and mutant full-length BCOR were efficiently expressed and immunoprecipitated, however only the wild type BCOR was able to coprecipitate full-length BCL6 (Figure 5A). BCOR has previously been shown to have repressive activity in two transient reporter assays: potentiation of repression by BCL6 and repression of AF9 activation. We tested the BCOR triple mutant in both assays and found that while it had identical activity as wild type BCOR in the AF9 assay, it failed to potentiate BCL6 repression (Figure 5 B, C). Because these experiments were carried out with full-length corepressor, our results support the presence of a single BCL6 binding site in BCOR. We conclude that the BCOR BBD is essential for the interaction with BCL6 and potentiation of its repression activity.

A BCOR^{BBD} peptide is a BCL6 inhibitor

The BCL6 BTB domain is required for its transcriptional repressor functions, and micromolar concentrations of a cell permeable TAT-SMRT^{BBD} peptide can effectively inhibit BCL6 (Polo et al., 2004). Since the BCOR and SMRT BBDs compete for a similar region of BCL6, we predicted that a cell permeable TAT-BCOR^{BBD} peptide would block the biological actions of BCL6 in B-cells. Accordingly, exposure of the BCL6 positive DLBCL cells Ly1, Ly7 and Ly10 to 5 μM TAT-BCOR^{BBD} fusion peptide de-repressed the BCL6 target genes CD69, CD80 and p21 (Figure 5D). In contrast, TAT-BCOR^{BBD} could not reactivate a negative control gene (CD20). The peptide did not activate any of these genes in a BCL6 negative DLBCL cell line (Ly4 cells). We previously showed that BCL6 is required for the survival of DLBCL cells (Polo et al., 2004; Polo et al., 2007), and accordingly, administration of TAT-BCOR^{BBD} peptides killed BCL6 positive DLBCL cells in a dose dependent manner, but had no effect on BCL6 negative DLBCL cells (Figure 5E). Therefore, the BCOR BBD abrogates the repressor activity of the BCL6 BTB domain and could serve as a structural template for development of targeted therapy for DLBCL.

DISCUSSION

Many regulated processes such as signal transduction and transcriptional regulation depend on interactions between evolutionarily conserved protein modules and short peptide motifs (Pawson and Nash, 2003). Protein domain families can often be further classified into subtypes according to their ligand binding specificity (Tong et al., 2002), and occasionally, a protein domain can bind to multiple classes of ligands. For example, the Fyn SH3 domain can bind to both type and I and type II polyproline ligands, and the selection is largely determined by the conformation of a conserved tryptophan at the binding site (Fernandez-Ballester et al., 2004).

In the case of BCOR and SMRT/NCoR binding to the BCL6 BTB lateral groove, distinct interactions are used to produce high selectivity for two dissimilar ligands. The lack of a consensus sequence between these two corepressor BBDs leaves open the possibility that additional peptides with no sequence relatedness to either the BCOR or SMRT BBDs may also bind to the BCL6 lateral groove, but these will have to be discovered experimentally. Recently, a 10 residue peptide aptamer was found to bind to the BCL6 BTB domain and reverse BCL6 activities (Chattopadhyay et al., 2006) but has no discernable sequence similarity to either the BCOR or SMRT/NCoR BBDs.

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The competitive nature of BCOR/SMRT/NCoR binding to BCL6 (Huynh et al., 2000) can be readily understood in light of the structural results presented here, since all three corepressor BBDs bind to the same surface of the BTB domain. However, the stoicheometry of the 2:2 BTB/BBD complex has important implications for the function of BCL6. The BTB domain is a highly stable homodimer, and the two equivalent BBD binding surfaces are formed at the interface of the BCL6 chains. Neither the BCOR or SMRT BBDs (this work and Ahmad et al., 2003) show cooperative binding. As a result, one BCL6 dimer can, in principle, bind simultaneously and independently to two different corepressors. An important consideration is thus the number of BCOR, SMRT or NCoR chains in the respective corepressor complexes. For example, if a BCL6-bound complex contains a single copy of the NCoR protein, the second binding site on BCL6 may still be available to interact with a second independent corepressor complex. If, however, a bound complex contains two or more corepressor chains, this single complex might be sufficient to saturate the available BCL6 BBD binding sites.

The potential for simultaneous recruitment of different corepressor complexes to regulatory sites has important implications for the mechanism of regulation of BCL6-dependent genes, since different corepressor complexes are associated with different chromatin modifying activities. SMRT and NCoR are found in histone deacetylase containing complexes (Guenther et al., 2000; Li et al., 2000; Wen et al., 2000), while the recently described BCOR complex contains both Polycomb group (PgG) proteins, including a histone H2A monoubiquitylation activity, and SCF E3 ligase components (Gearhart et al., 2006). Our structural characterization of the binding modes of the BCOR and SMRT corepressors to the BCL6 lateral groove provides crucial information for the rational design of improved inhibitors for the treatment of BCL6-dependent DLBCL.

EXPERIMENTAL PROCEDURES

See the Supplemental Data for additional experimental procedures.

PAGE Binding Assays

For native PAGE band shift assays, 4.5 μl of BCL6^{BTB} (2.5 mg/ml) was mixed with 1 μl of either Thioredoxin-corepressor fragment fusion proteins (4 mg/ml), 4.5 μl of distilled water and 2 μl of sample buffer. Samples were incubated at 4° C for 20 minutes and analyzed by 4% PAGE. Proteins were visualized by Coomassie blue staining.

Crystallization

A four -fold molar excess of BCOR^{BBD} (residues 498–514) was mixed with BCL6^{BTB} (residues 5–129) and concentrated to a final concentration of 10 mg/ml in 150 mM NaCl, 20 mM Tris-HCl (pH 8.1) and 1 mM (*tris*(2-carboxyethyl)phosphine (TCEP). Crystals were obtained by hanging drop vapor diffusion at room temperature by mixing 1 μl of the protein solution with 1 μl of reservoir solution (75 mM Na acetate pH 4.5, 900 mM K_2HPO_4 and 700 mM NaH_2PO_4). Crystals formed in space group $P6_122$, with unit cell dimensions a,b = 150.55 Å, c = 312.25 Å. The atomic coordinates and structure factor data for the BCL6^{BTB}/BCOR^{BBD} complex have been deposited with the Protein Data Bank (accession code 3BIM).

Fluorescence Polarization

A modified SMRT peptide containing a C terminal cysteine and a H1426W mutation was labeled with Alexa Fluor 488 C5 maleimide (A488-SMRT) and purified by hplc. Assays were performed in 96-well black bottom Microfluor-1 plates on a Molecular Devices SpectraMax M5 reader using excitation and emission wavelengths of 495 nm and 540 nm, respectively. For the binding assay, each well contained 300 μL of 20 mM Tris pH 8.3, 150 mM NaCl, 1 mM TCEP buffer and 5 nM A488-SMRT. For the competitive displacement experiments, 5

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nM A488-SMRT and 3 μM $BCL6^{BTB}$ were used in all samples with increasing concentration of competitor peptides. The data were analyzed as described in the Supplemental Data.

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Supplementary Material

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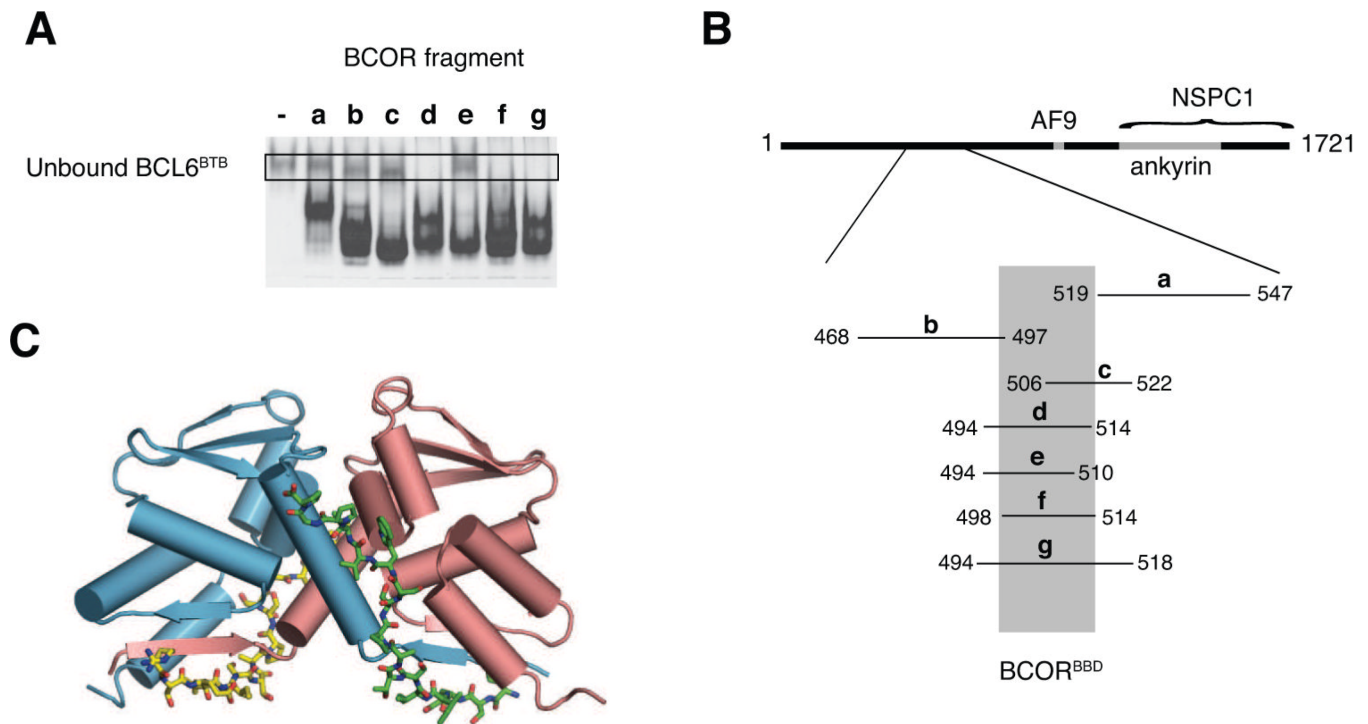

Figure 1. Identification of the BCOR BCL6 Binding Domain (BCOR^{BBD})

(A) BCOR fragments were purified as thioredoxin (Trx) fusion proteins and assayed for binding to the BCL6 BTB domain by native gel electrophoresis. The horizontal box indicates the position of unbound BCL6 BTB. BCL6^{BTB} was loaded alone (lane "-") or as a mixture with the Trx-BCOR fragments described in panel B. Equal amounts of BCL6^{BTB} were loaded in all lanes. (B) Schematic of the long-isoform BCOR protein, indicating the fragments used in the binding assay in panel (A). (C) Crystal structure of the BCL6^{BTB}/BCOR^{BBD} complex. The BCL6^{BTB} dimer is shown in cartoon representation with blue and pink subunits. The two bound BCOR^{BBD} peptides are shown in bond representation with green and yellow carbons, respectively.

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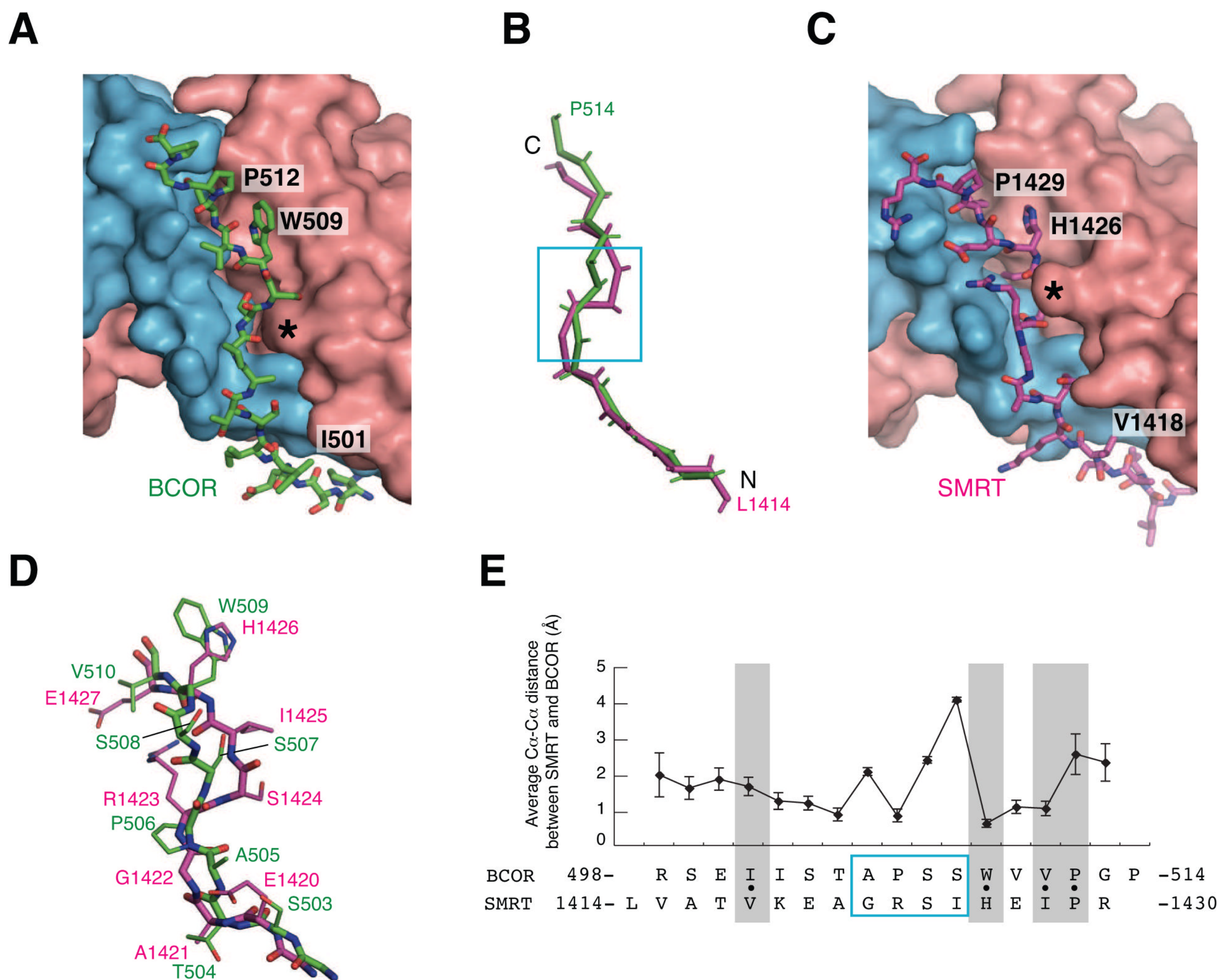

Figure 2. Comparison of the BCOR and SMRT BBD complex structures

(A) View of the lateral binding groove in the $BCL6^{BTB}/BCOR^{BBD}$ complex. The BTB dimers are shown as solvent accessible surfaces with subunit coloring as in Figure 1C. The asterisk indicates the position of residue H116 from BCL6, which is buried beneath the BBD. (B) Superposition of $BCOR^{BBD}$ (green) and $SMRT^{BBD}$ (red) peptides, represented as Cα traces with added Cβ positions. The boxed region indicates BCOR residues A505-S508 and SMRT residues G1422-I1425, in which the BBDs adopt significantly different conformations. (C) The $BCL6^{BTB}/SMRT^{BBD}$ complex (Ahmad et al., 2003). In this case, BCL6 residue H116 (asterix) covers $SMRT^{BBD}$ residues I1425 and S1424. (D) Expanded view of the central region of the superposed BCOR and SMRT BBDs. (E) Structure-based sequence alignment of the BCOR and SMRT BBDs. Grey shading indicates the four residues that form similar side chain contacts with the BCL6 BTB domain. These are the only positions with sequence similarity (I/V; W/H) or identity (Pro) between the BCOR and SMRT BBDs. The graph shows the variation in the average Cα position between the BCOR and SMRT peptides. The error bars are the standard deviation of the 16 independent measured distances, based on two independent SMRT and eight independent BCOR crystallographic observations of the peptide structures.

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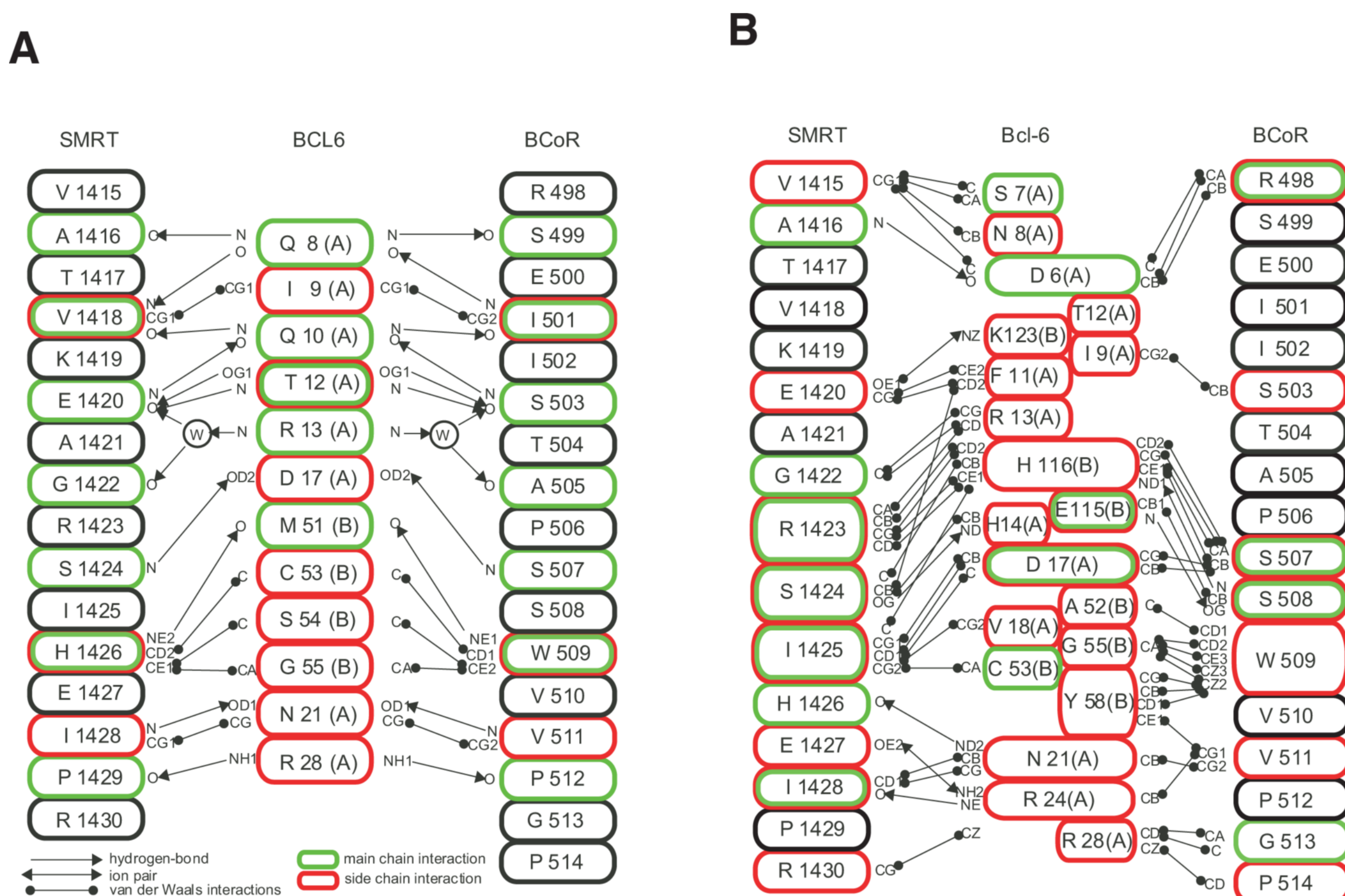

Figure 3. Contacts between BCL6 and the SMRT and BCOR BBDs

(A) Schematic representation of conserved BCL6 contacts in the BCOR and SMRT BBDs. The complete BBD sequences are shown, while only the BCL6 residues involved in BBD contacts are shown. Green and red ovals highlight the residues participating in main chain and side chain contacts, respectively. Black ovals enclose the residues not forming conserved contacts. A conserved water at the peptide-protein interface near BCL6 Arg13 is indicated as an encircled “W”. Most of the contacts are polar and involve main chain atoms from the BBD peptides. (B) Similar to panel (A), but showing non-conserved interactions. In this case, most of the contacts are non-polar van der Waal packing interactions involving BBD side chains.

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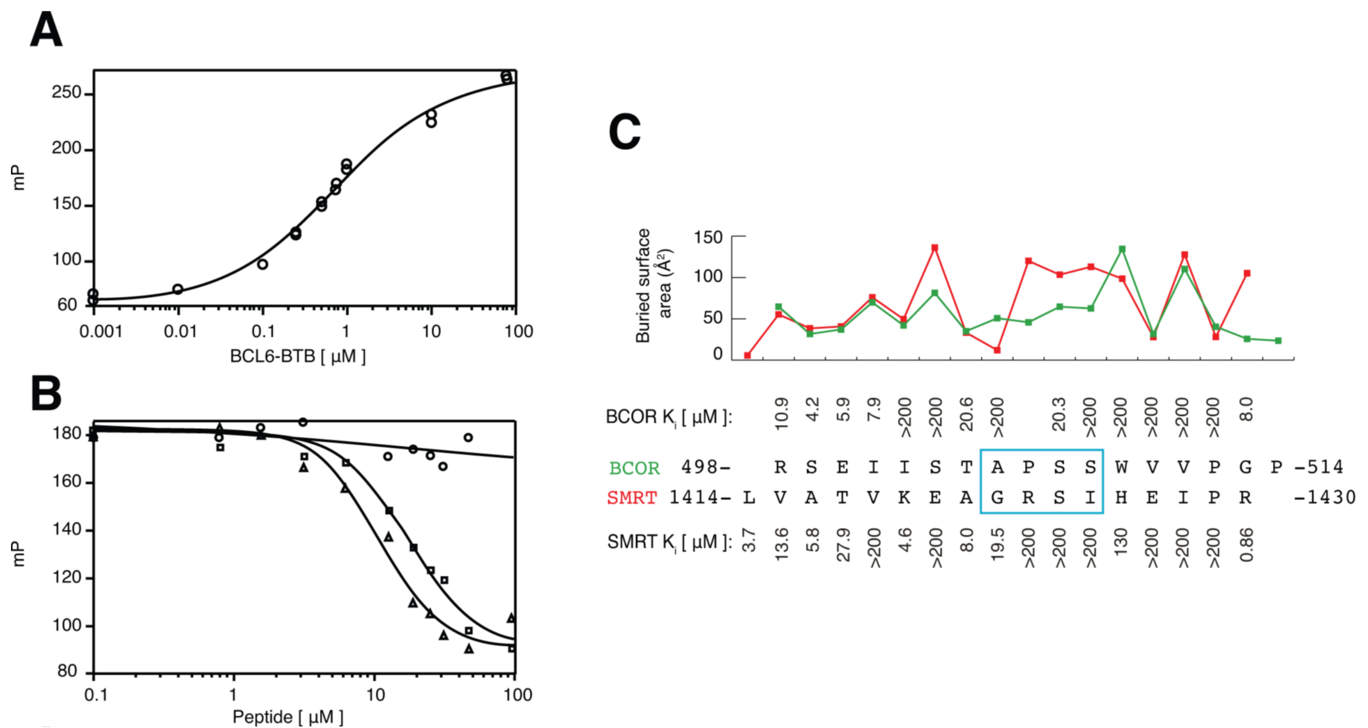

Figure 4. Alanine scan of the BCOR and SMRT corepressor peptides

(A) Fluorescence Polarization (FP) binding curve for A488-SMRT as a function of the $BCL6^{BTB}$ concentration. (B) Representative competition binding curves in which unlabelled peptides are added to disrupt the $BCL6^{BTB}$/A488-SMRT complex. Each residue in $BCOR^{BBD}$ or $SMRT^{BBD}$ was replaced by an alanine (or glycine where the native residue is an alanine) in a series of non-fluorescent synthetic peptides. Triangles: $SMRT^{BBD}$, squares $BCOR^{BBD}$, circles: $BCOR^{BBD}$ W509A. The complete set of titrations are included in Figures S7 and S8. (C) The Ki values (μM) derived from the FP competition curves for the single residue Ala or Gly substituted $BCOR^{BBD}$ and $SMRT^{BBD}$ peptides are indicated below a plot of the average buried surface area of the residues of the $BCOR^{BBD}$ (green) and $SMRT^{BBD}$ (red) peptides in the crystal structure.

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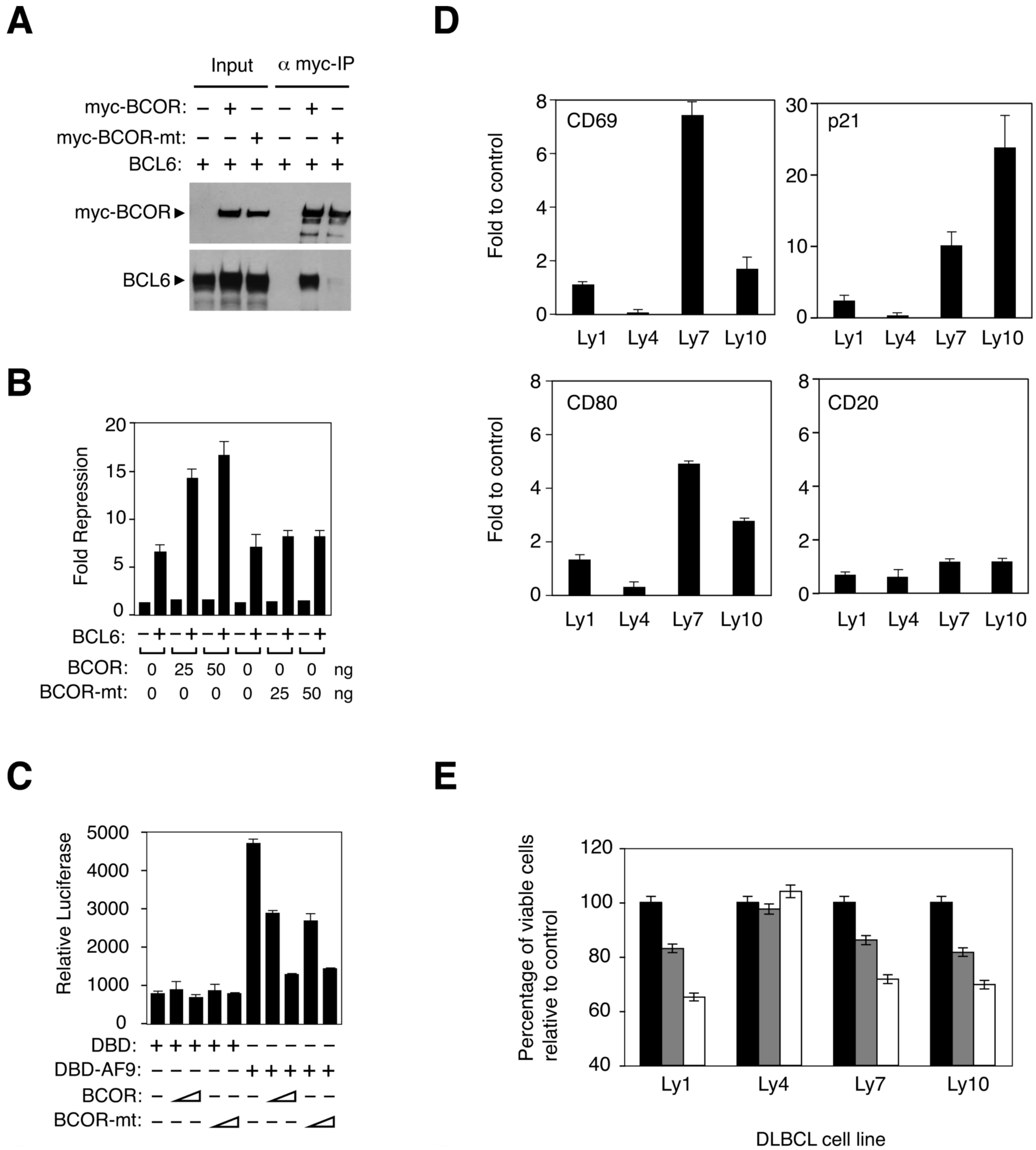

Figure 5. Mutation of the BCOR BBD specifically affects its ability to function with BCL6

(A) BCOR BBD mutations abrogate the interaction of BCOR with BCL6 in vivo. HEK293 cells were transfected with 0.1 μg each of BCL6 expression plasmid alone or together with 0.5 μg myc-BCOR or myc-BCOR-mt (BCOR S507A/W509A/V511A mutant). Cell lysates were immunoprecipitated with α-myc antibody and recovered proteins were detected by western blotting using N-3 α-BCL6 and 9E10 α-myc antibodies. (B) Mutation of the BCOR BBD abrogates the ability of BCOR to potentiate BCL6 repression. (C) Mutation of the BCOR BBD does not affect the ability of BCOR to repress AF9 activation. (D) BCL6 positive (Ly1, 7 and 10) and BCL6 negative (Ly4) diffuse large B-cell lymphoma cell lines where exposed to 5 μM TAT-BCOR^{BBD} or a control peptide. Levels of BCL6 target genes CD69, p21 and CD80

as well as the negative control (non-BCL6 target gene) CD20 were measured by QPCR. (E) Ly1, 4, 7 and 10 cells were allowed to grow for 48 hours in the presence of no added BCOR inhibitor peptide (black bars); 2 μM peptide (grey bars); or 5 μM peptide (open bars). Error bars in panels B-E indicate the standard error.

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