CHAPTER 9

Structural Determinants of CtBP Function

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Abstract

The structural characteristics of the CtBP family of transcriptional corepressors suggest an additional role for coenzyme nicotinamide adenine dinucleotide in the repression of gene expression. Remarkably, CtBP orthologues are unique among transcriptional regulators in that they display striking primary sequence and structural similarity to the D-isomer specific 2-hydroxyacid dehydrogenase class of enzymes. Recent structural studies of rat CtBP/BARS and human CtBP1 provide insight into the role of pyridine dinucleotide binding in regulation of CtBP quaternary structure, and corepression activity through association with –PXDLS-containing targets.

Introduction

In addition to a central essential role in metabolism as a carrier of reducing equivalents, the nicotinamide adenine dinucleotide coenzymes (NAD and NADP)[a] play important roles in cellular signaling, also serving as substrates for covalent protein modifications as well as precursors to the synthesis of intracellular calcium mobilizing second messenger molecules (reviewed in ref. 1). Moreover, recent studies implicate NAD in a variety of nuclear transactions. For some of these processes, a role for NAD as a recipient or donor of reducing equivalents seems to be important. For example the DNA binding of Clock-BMAL1 and NPAS2-BMAL1 heterodimers is regulated by the ratio of reduced to oxidized NAD(P).[2,3] In contrast, other NAD-dependent nuclear processes result in a net consumption of NAD, cleaving the N-glycosidic bond between nicotinamide and ADP-ribose. DNA damage response pathways activate poly(ADP-ribose) polymerase-1 (PARP-1) leading to the addition of polymers of ADP-ribose to a number of nuclear proteins.[4] In another example, the chromatin-associated silencing protein Sir2 (Silencing information regulator 2) functions as a NAD-dependent protein deacetylase acting on histones as well as a variety of transcription factors, generating O-acetyl-ADP-ribose and nicotinamide as products.[5]

The structural characteristics of the CtBP family of transcriptional corepressors suggest an additional role for the coenzyme NAD in transcriptional regulation. The first CtBP homologue (CtBP1) was identified as a binding partner for the adenoviral transforming protein E1A[6] however CtBP homologues have been implicated as cofactors for a number of cellular

[a] By convention, NAD(P) refers to nicotinamide adenine dinucleotide (phosphate) without reference to the dinucleotide oxidation-reduction state. $NAD(P)^+$ specifies the oxidized form, whereas NAD(P)H specifies the reduced form.

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transcriptional repressors.[7,8] CtBP target proteins share a common consensus motif, related to -PXDLS-, which is essential for the recruitment of CtBP. Deletion of this motif in these proteins abrogates CtBP binding and, at least partially, their transcriptional repressor activities. Remarkably, CtBP orthologues lack prototypical features associated with typical transcriptional regulatory proteins, but are unique among transcriptional regulators in that they display striking primary sequence and structural similarity to the D-isomer specific 2-hydroxyacid dehydrogenase class of enzymes. These proteins are also unusual in that CtBP homologues appear to harbor dual cytoplasmic and nuclear functions. In addition to a proposed widespread roles in transcription, nonnuclear functions of CtBP homologues are suggested by the recognition that the Brefeldin A ribosylation substrate or BARS-50, a component of the Golgi tubule fission complex, is virtually identical to CtBP1.[9] The identification of RIBEYE, a CtBP2 splice variant as a component of the ribbon synapse[10] additionally supports the concept of functional complexity beyond the nucleus for this family of proteins.

What is the significance of the dehydrogenase homology in the transcriptional repression functions of CtBP? Current studies suggest two general models. In the first, the dehydrogenase domain serves a structural or scaffolding function independent of an enzymatic activity for the recruitment of other coregulatory proteins, including proteins with chromatin remodeling activities. In support of this mechanism, numerous laboratories have found an association of CtBP1 and CtBP2 with histone deacetylases (reviewed in ref. 8), and a macromolecular corepressor complex comprised of CtBP in association with histone deacetylase, histone methyltransferase and demethylase activities as well as other corepressors has been purified from HeLa cells.[11] Notably, dinucleotide promotes the interaction of CtBP with E1A and other targets[12-14] and facilitates the dimerization of CtBP.[15,16]

An alternative but not necessarily exclusive model is that CtBP homologues harbor an intrinsic enzymatic activity essential for these diverse functions. Although several laboratories have demonstrated weak dehydrogenase activity with a surrogate substrate, the identities of bona fide substrate(s) for any oxidation-reduction enzymatic activity of CtBP remain elusive. Consequently, how CtBP might participate as a dehydrogenase in the process of transcriptional repression remains speculative. Moreover the link between a putative dehydrogenase activity and one activity proposed for CtBP/BARS, an acyltransferase activity employing acyl-CoA in the conversion of lysophosphatidic acid into phosphatidic acid[17] is unclear. In this chapter, we explore the structural features of CtBP and the relationship to the known biochemical properties of this unusual corepressor protein family.

CtBP: General Structural Considerations

Schaeper et al[6] first noted the similarity of CtBP to the D-isomer specific 2-hydroxyacid dehydrogenases with the cloning of CtBP1. This family of proteins, with members conserved from prokaryotes to higher metazoan organisms, includes formate dehydrogenase (FDH), D-glycerate dehydrogenase (D-GDH), D-3-phosphoglycerate dehydrogenase (3PGDH), D-lactate dehydrogenase (D-LDH), and D-2-hydroxyisocaproate dehydrogenase (D-HicDH).[18] These enzymes are typically composed of 2 to 4 identical polypeptide subunits, each subunit composed of 2 or more domains.

To date, three crystallographically determined structures of CtBP homologues have been reported, including a the structure of a core domain of human CtBP1 refined to 1.95 angstrom (Å) resolution (Protein Data Bank entry 1MX3),[13] and the closely related rat CtBP/BARS protein in complex with NAD at 2.3 Å resolution (Protein Data Bank entry 1HKU).[14] The latter report also includes the solution of a ternary complex of CtBP/BARS with NAD and a model PXDLS peptide (PIDLSKK) at 3.1 to 3.5 Å resolution (Protein Data Bank entry 1HL3) (Fig. 1). Human CtBP1 and rat CtBP/BARS have a high degree of homology (97% identity), differing in sequence primarily due to an amino terminal extension of CtBP1 not present in CtBP/BARS.[9,14] Each of these structures share overall structural homology with the core domains of the D-2-hydroxyacid dehydrogenases.