CHAPTER 8

CtBP Corepressor Complex - A Multi-Enzyme Machinery that Coordinates Chromatin Modifications

Yu-Jiang Shi and Yang Shi*

Abstract

Recent biochemical and proteomic approach has identified a CtBP super complex consisting of a host of chromatin modifying enzymes. Analysis of this complex has led to the appreciation that enzymes that mediate deacetylation and histone H3 lysine 9 methylation are present in the same biochemical complex, which facilitates coordinated histone modifications important for establishing repressive chromatin. Importantly, studies of this complex also resulted in the finding of the first histone demethylase LSDI, which represses transcription by demethylating histone K4, where methylation is linked to active transcription. It is anticipated that additional important new insights will be gained from further investigation of this unusual transcriptional repression machine.

CtBP is a transcriptional corepressor and is one of the three main transcriptional cofactors that are directly targeted by the viral oncoprotein E1A during oncogenic transformation.[1] To explore mechanisms by which CtBP mediates transcriptional repression, a biochemical approach was taken to isolate proteins that are associated with CtBP. This effort has led to the identification of a CtBP super-complex, consisting of, among others, six potential enzymatic activities.[2] While the exact composition of this super-complex may differ in different cell types, characterization of these enzymatic functions in HeLa cells has already provided significant insight into mechanism of action of CtBP and eukaryotic gene regulation. Below we provide a brief discussion of the enzymatic components of the CtBP complex and our current understanding of their individual as well as coordinated enzymatic actions in transcriptional repression. While other aspects of CtBP are covered in other chapters, this chapter is largely confined to the CtBP super complex.

Six Enzymes/Potential Enzymes in the CtBP Super-Complex

CtBP: A Nuclear Dehydrogenase

CtBP shares significant sequence homology with members of the dehydrogenase family, in particular the 2-hydroxy acid dehydrogenases,[3] but this predicted enzymatic activity has not been demonstrated experimentally until recently.[2,4,5] Using pyruvate as a substrate, NADH as a cofactor and bacterially purified CtBP1 as the source of enzyme, studies from a number of labs have shown that CtBP1 can convert pyruvate to lactate, as measured by the conversion of NADH to NAD in a dehydrogenase reaction. Importantly, mutation of the conserved histidine

*Corresponding Author: Yang Shi—Department of Pathology, Harvard Medical School, Boston, Massachusetts, U.S.A., Email: yang_shi@hms.harvard.edu

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residue (amino acid 315), predicted to be critical for catalysis, completely abolished the dehydrogenase activity, strongly suggesting that this newly found enzymatic activity is intrinsic to CtBP1. X-ray crystallography provided further structural insight that substantiates the notion that CtBP1 is a dehydrogenase.[5,6] However, two important issues remain. First, the Km for CtBP-mediated dehydrogenation reaction is significantly higher than any bona fide dehydrogenases. It is therefore almost certain that pyruvate is not the physiological substrate of the CtBP proteins. It will be important to identify the physiological substrate, which constitutes an important piece of the missing puzzle. Second, the biological significance of this dehydrogenase activity is still unclear. Using mouse embryonic cells (MEF) carrying null alleles for both CtBP1 and CtBP2, Frisch, Goodman and colleagues found that the catalytically inactive CtBP mutant functions like the wildtype protein in its ability to restore repression of the CtBP target genes in vivo.[7] This finding suggested strongly that the dehydrogenase activity is not required, at least not directly, for CtBP-mediated transcriptional repression. A recent study of the drosophila CtBP suggests that NAD binding is important for CtBP-mediated repression after CtBP is recruited to the promoter.[8] Thus, it remains a challenge to decipher the role of the dehydrogenase activity of CtBP in vivo.

HPC2: A Chromodomain-Containing Sumo E3 Ligase that May Bind Methylated K27 of Histone H3

HPC2 is the human homolog of the Drosophila Polycomb (Pc) protein, which is a part of the Polycomb Group (PcG) protein complex. HPC2 has been found in the CtBP super complex[2] and has also been isolated as a CtBP-interacting protein in a separate two-hybrid screen.[9] HPC2 contains the CtBP-interacting PXDLS motif, suggesting that HPC2 directly interacts with CtBP. Recent studies showed that HPC2 is a Sumo E3 ligase for CtBP, and that CtBP sumoylation is important for CtBP nuclear localization.[10] It is unknown whether HPC2-mediated sumoylation also impacts other aspects of the CtBP complex, in addition to CtBP subcellular localization. HPC2 has also been implicated in tumorigenesis based on the observation that over-expression of HPC2 C-terminal deletion mutants induce oncogenic transformation and apoptosis.[11] It has been hypothesized that these mutants transform cells by interfering with the activity of the endogenous HPC2. It will be interesting to explore whether the transforming activity of the mutant HPC2 proteins is linked to the disruption of the Sumo E3 ligase activity of HPC2, which would be predicted to effect CtBP subcellular localization and probably CtBP complex formation as well. Lastly, HPC2 also contains a chromodomain located N-terminally. The prototypical chromodomain, as exemplified by HP1 protein, has been shown to bind methylated H3-K9. HP-1, together with the heterochromatin-specific H3-K9 methylase Suv-39, is believed to be responsible for heterochromatin propagation.[12-14] The Drosophila Pc protein binds tri-methylated histone H3 lysine K27.[15-17] Whether the chromodomain in human PC2 plays a similar role to coordinate CtBP-mediated transcriptional repression remains to be determined.

HDAC1/2 and G9a/HMTase1: Histone Deacetylases and Methylases

The class I histone deacetylase HDAC1 and 2 as well as the two highly related euchromatic histone methylases G9a and HMTase1[18] have also been identified as components of the CtBP1 complex. Interactions of CtBP with HDAC1 and 2 have also been reported by other studies.[19-21] While HDAC1/2 can mediate deacetylation reactions on a host of lysine (K) residues on histones, G9a/HMTase function as heterodimer, which mediates methylation of histone H3, predominantly on lysine 9 (H3-K9) and, to a lesser extent, on lysine 27 (H3-K27).[18,22] K9 is a critical amino acid residue on the tail of histone H3 where different modifications are correlated with different transcriptional activity. Specifically, H3-K9 acetylation is associated with active transcription while methylation at the same site is associated with heterochromatin or repressive euchromatin.[23] The fact that both histone deacetylases and methylases are present in the CtBP repressor complex suggests that CtBP may be able to convert an active chromatin