# CHAPTER 7

# CtBP as a Redox Sensor in Transcriptional Repression

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## Abstract

The corepressor CtBP (carboxyl-terminal binding protein) is involved in transcriptional pathways important for development, cell cycle regulation, and transformation. We demonstrate that CtBP binding to transcription repressors is stimulated by $NAD^+$ and NADH, with NADH being two to three orders of magnitude more effective. Fluorescence resonance energy transfer studies of CtBP show a >100-fold higher affinity for NADH than $NAD^+$, in agreement with the tighter interaction observed in the crystal structure of NADH-bound CtBP. Levels of free nuclear nicotinamide adenine dinucleotides, determined using two-photon microscopy, correspond to the concentrations required for half-maximal CtBP binding. Free cellular $NAD^+$ concentration greatly exceeds that of NADH and the redox changes are mainly reflected by NADH levels. Agents increasing NADH levels stimulate CtBP binding to its partners in vivo and potentiate CtBP-mediated repression. These findings suggest that the transcriptional corepressor CtBP may serve as a redox sensor to provide a link between gene expression and metabolism.

## Introduction

The metabolic state of a cell is thought to influence cellular functions including transcription. Recently, a few connections have been made between changes in metabolic state and effects on gene regulation through the action of nicotinamide adenine dinucleotides. The focus of this chapter is the regulation of gene expression by the metabolic state of the cell through the electron carrier redox pair $NAD^+$/NADH and the ability of the transcriptional corepressor CtBP to serve as a redox sensor. We hypothesize that CtBP provides an important link between gene expression and metabolism.

## Differential Binding of CtBP to $NAD^+$ and NADH

The carboxyl terminal binding protein (CtBP) is a transcriptional corepressor important for development, cell cycle regulation, and transformation.[1] CtBP was first identified through its ability to interact with the carboxyl terminus of adenovirus E1A oncoprotein.[2] The residues in the carboxyl terminus critical for the interaction were determined to be the PLDLS sequence.[3]

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*CtBP Family Proteins*, edited by G. Chinnadurai. 

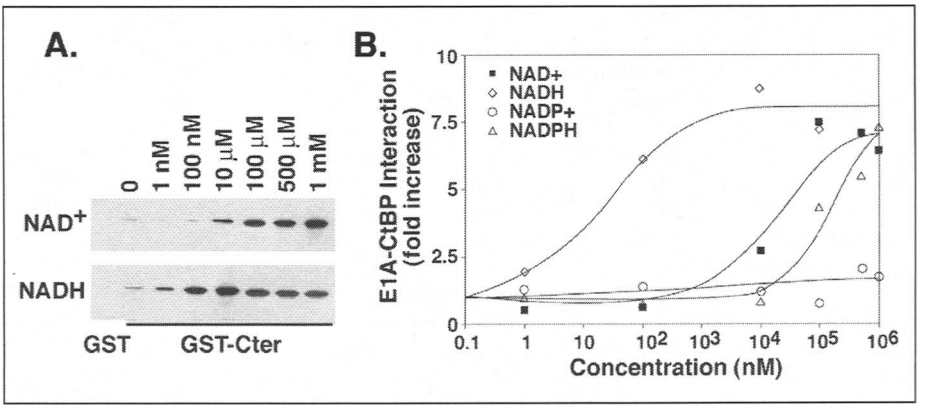


Figure 1. A) Binding of recombinant CtBP to GST-E1A at various concentrations of $NAD^+$ and NADH. B) Relative interactions as a function of nicotinamide adenine dinucleotide concentration depicted on a log scale.

The dehydrogenase domains of CtBP and 3-phosphoglycerate dehydrogenase are nearly 40% identical. A weak dehydrogenase activity has been reported for CtBP in the presence of pyruvate and NADH,[4-6] but the turnover rate of CtBP is 30,000-fold less than that of lactate dehydrogenase.[7] Furthermore, the enzymatic activity of CtBP, which can be blocked by mutation of the catalytic center His 315, is not required for repression of target genes in CtBP-knockout mouse embryo fibroblasts.[8] Previous transcriptional tethering studies have also shown that the His residue of the catalytic triad is dispensable for the transcriptional repressive activity of mCtBP2[9] and dCtBP.[10] This suggests that the dehydrogenase activity is not essential for CtBP-mediated gene repression.

Nonetheless, it is possible that CtBP, like the dehydrogenases and reductases, is regulated by $NAD^+$ or NADH in some other capacity. One model that we considered was that $NAD^+$/NADH could affect the ability of CtBP to interact with its partners. To test this hypothesis, we examined the interaction of bacterially-expressed CtBP with GST-E1A fusion proteins at different concentrations of $NAD^+$/NADH. To our surprise, CtBP binding was regulated dramatically, with NADH increasing the interaction at concentrations in the nM range (Fig. 1). $NAD^+$ also increased binding, but was two-to-three orders of magnitude less effective. $NADP^+$ and NADPH had little if any effect (Fig. 1).

Because $NAD^+$/NADH similarly affected CtBP binding to a variety of transcriptional repressors, we speculated that the nicotinamide adenine dinucleotides functioned by altering CtBP structure. Support for this idea was obtained from limited proteolysis assays demonstrating an induced conformational change of CtBP upon $NAD^+$/NADH binding.[11] This observation agrees with numerous studies of dehydrogenase structure and function. In general, nucleotide binding to dehydrogenase proteins induces a conformational change involving the movement of a flexible loop over the active site, thus favoring catalysis by optimizing the positions of catalytic and substrate binding residues. NADH protects CtBP from the proteolytic digestion at a lower concentration than $NAD^+$.[11]

Regulation of CtBP binding to its partner by $NAD^+$/NADH has attracted much attention and is somewhat controversial. Although the ability of nicotinamide adenine dinucleotides to stimulate CtBP binding to E1A and other proteins has been confirmed by several laboratories,[4,5] the differential efficacy of $NAD^+$ and NADH has been challenged. We showed that NADH was two-to-three orders of magnitude more effective than $NAD^+$ in stimulating CtBP binding and proposed that this differential effect might link CtBP-mediated repression to the redox state of the nuclear compartment. In contrast, Kumar et al[4] and Balasubramanian et al[5]