CHAPTER 2

Transcriptional Repression by the CtBP Corepressor in *Drosophila*

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Abstract

Transcriptional repression is essential for patterning gene expression in the early *Drosophila* embryo. Biochemical and genetic studies on *Drosophila* C-terminal binding protein (dCtBP) have provided solid evidence that dCtBP acts as a corepressor for several transcriptional repressors. Similarly to mammalian CtBPs, dCtBP interacts with a short peptide motif, PxDLS, or related motifs. It appears that dCtBP is essential for short-range transcriptional repression in the early embryo. In contrast, it has been recently reported that dCtBP participates in Polycomb-mediated long-range repression. In this chapter, we will review how the dCtBP corepressor functions, from the biochemical, developmental, and genetic point of views.

Introduction

Numerous biochemical and genetic analyses have established *Drosophila melanogaster* (fruit fly) as one of the most accessible model systems for studying transcriptional networks, regulatory elements and factors controlling them. During early *Drosophila* embryogenesis, a hierarchy of gene networks consisting of maternal and zygotic genes (gap, pair rule, segmentation polarity genes, etc.) progressively divides the embryo into increasingly precise segments/territories.[1,2] This patterning process further depends on broadly distributed activators and localized sequence-specific repressors to refine the initial segmentation boundaries.

In 1995, Chinnadurai and colleagues cloned the human *CtBP1* (*hCtBP1*) gene. hCtBP1 interacts with the adenovirus E1A oncoprotein through a specific amino acid motif, PLDLSCK.[3] In 1998, using yeast two-hybrid screens, two laboratories identified dCtBP as a factor that physically interacted with three transcriptional repressors involved in embryonic patterning: Knirps, Snail, and Hairy.[4,5]

Structure of the *dCtBP* Gene and Its Proteins

Drosophila carries a single copy of the *dCtBP* gene on the right arm of the third chromosome (located cytologically at 87D8-87D9),[4-6] whereas human and mouse have two highly related CtBP genes, CtBP1 and CtBP2.[7,8]

The annotation of the *dCtBP* gene, based on the analyses of both *dCtBP* expressed sequence tag (EST) clones and the fly genome sequence, predicts 386 amino acids (aa) and four splicing variants differing in 5' untranslated region of the mRNA (Fig. 1A).[6] Due to heterogeneity of the 5' termini, *dCtBP* was predicted to be transcribed by four separate promoters. In protein

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CtBP Family Proteins, edited by G. Chinnadurai.

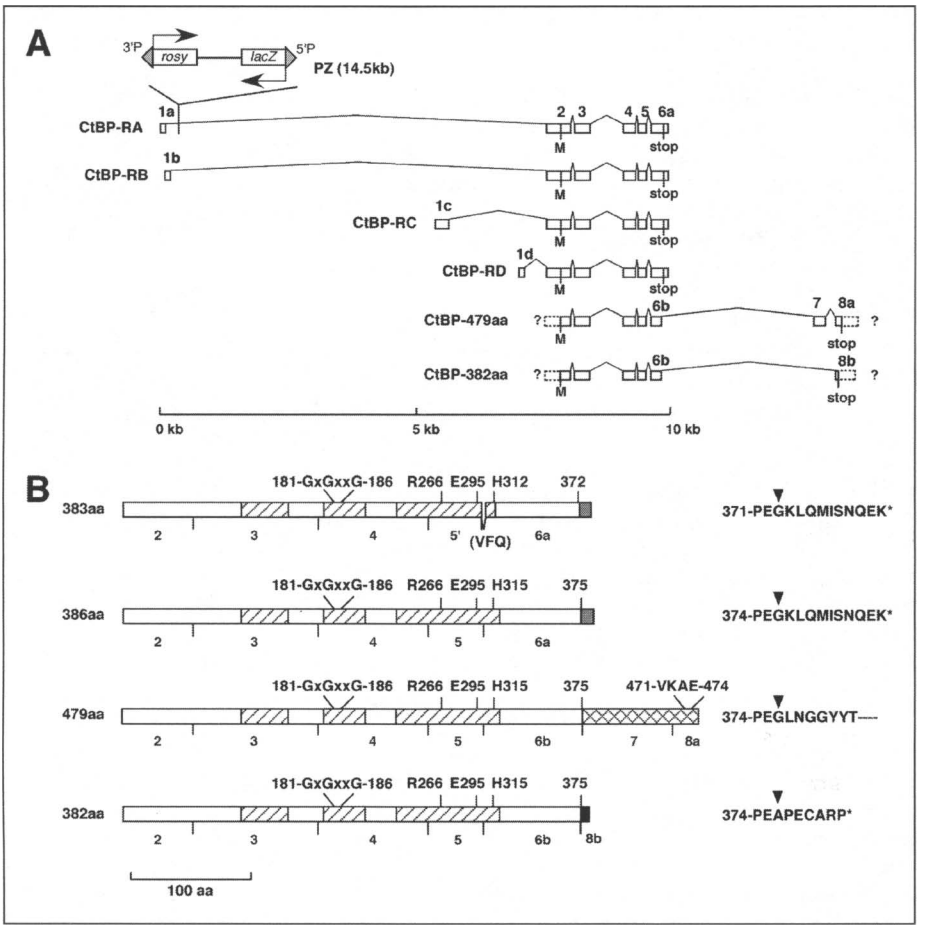

Figure 1. Structure of the *dCtBP* gene and the dCtBP protein isoforms. A) Organization of the *dCtBP* transcription unit. Each exon is shown as an open box and assigned a number on top. Introns are indicated by thin lines. Locations of the translation initiation site and the stop codon are indicated by "M" and "stop", respectively. Four transcripts, CtBP-RA to -RD, are annotated in the fly base and all encode the 386aa isoform.[4,5] CtBP-479aa and CtBP-382aa are also shown in this panel based on the previous studies and our unpublished results, but the exon/intron structures 5' of the translation initiation and 3' of the stop codon are unknown. The insertion of the PZ P-element (14.5 kb) located 500 bp downstream of exon 1a in the *dCtBP*^{03463} mutant disrupts dCtBP function. B) Schematic structure of dCtBP proteins. The NAD^+/NADH binding motif (GxGxxG) and the catalytic triad (arginine, glutamic acid, and histidine) are conserved among all the dCtBP isoforms. Hatched boxes indicate regions of high similarity with the dehydrogenases. Numbers and thin lines below each rectangle indicate exons. Shaded, double hatched, and solid boxes at the C-termini represent portions of splicing variants. The amino acid sequences around the alternative splicing points are shown right to the panel. Asterisks indicate the C-terminus ends. The splicing points (arrowheads) are G376 in dCtBP 479aa or A376 in the 382aa isoform. The 383aa isoforms derive from a shorter exon 5, lacking three amino acids, VFQ. "VKAE" in the 479aa isoform is a similar motif to the sumoylation site.

coding regions, at least four alternatively spliced forms obtained from the yeast two-hybrid screens, 382aa, 383aa (accession number AB011840), 386aa (accession number AJ224690), and 479aa, have been reported (Fig. 1B).[4,5,9,10] The CtBP family proteins are similar to