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MECHANISMS OF DEVELOPMENT

CtBP is required for proper development of peripheral nervous system in *Drosophila*

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ABSTRACT

C-terminal binding protein (CtBP) is an evolutionarily and functionally conserved transcriptional corepressor known to integrate diverse signals to regulate transcription. *Drosophila* CtBP (dCtBP) regulates tissue specification and segmentation during early embryogenesis. Here, we investigated the roles of dCtBP during development of the peripheral nervous system (PNS). Our study includes a detailed quantitative analysis of how altered *dCtBP* activity affects the formation of adult mechanosensory bristles. We found that *dCtBP* loss-of-function resulted in a series of phenotypes with the most prevalent being supernumerary bristles. These *dCtBP* phenotypes are more complex than those caused by Hairless, a known dCtBP-interacting factor that regulates bristle formation. The emergence of additional bristles correlated with the appearance of extra sensory organ precursor (SOP) cells in earlier stages, suggesting that dCtBP may directly or indirectly inhibit SOP cell fates. We also found that development of a subset of bristles was regulated by dCtBP associated with U-shaped through the PxDLS dCtBP-interacting motif. Furthermore, the double bristle with sockets phenotype induced by *dCtBP* mutations suggests the involvement of this corepressor in additional molecular pathways independent of both Hairless and U-shaped. We therefore propose that dCtBP is part of a gene circuitry that controls the patterning and differentiation of the fly PNS *via* multiple mechanisms.

1. Introduction

Proper patterns of gene expression are essential for animal morphogenesis. In many instances, broadly based signaling cues, such as morphogen gradients, initiate the formation of boundaries during development. Subsequently, the integrated function of both positive and negative regulators acts to refine previously established patterns. These reiterative mechanisms are abundantly represented in development. Pattern formation often requires transcriptional repression, as in the case of the formation of stripes and broad bands along both the anteroposterior and dorsoventral axes in the early *Drosophila* embryo (Ip and Hemavathy, 1997; Mannervik et al., 1999). The transcriptional corepressor *Drosophila* CtBP (dCtBP) is known to interact with several DNA-binding repressors through short peptide PxDLS motifs to establish a subset of sharply defined patterns of gene expression in the early fly embryo.

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CtBP belongs to the CtBP/BARS/RIBEYE/AN superfamily, and has been characterized as a transcriptional corepressor that is evolutionarily conserved from nematodes to humans (Chinnadurai, 2007; Stern et al., 2007). Unlike vertebrates, which contain up to three *CtBP* genes, *Drosophila melanogaster* has only a single copy of *dCtBP*, whose mRNA can be differentially spliced to produce at least four different isoforms (Mani-Telang and Arnosti, 2007; Nibu et al., 1998a, 1998b; Poortinga et al., 1998; Stern et al., 2007; Sutrias-Grau and Arnosti, 2004). dCtBP, together with short-range DNA-binding repressors, quenches only nearby DNA-binding activators, such as Bicoid and Dorsal, to establish additive patterns of gene expression (Nibu et al., 2003, 1998a,b).

The fruit fly peripheral nervous system (PNS) cell lineage is an excellent system for understanding pattern formation and cell fate specification (Bardin et al., 2004; Calleja et al., 2002; Gomez-Skarmeta et al., 2003). The formation of sensory organs, consisting of large sensory bristles (also called macrochaetes), is spatially and temporally controlled during the development of wing imaginal discs, the anlage for both the adult wings and the dorsal thorax. Specification of the PNS initiates when 20–30 cells of proneural clusters (PNCs) expressing the *achaete* and *scute* genes, which encode basic region-helix–loop–helix (bHLH) transcription factors, arise at certain positions in the monolayer of epidermal cells at the third-instar larval stage. This initial constellation of PNCs constitute a "pre-pattern", that is dictated by discrete enhancers that direct the spatially resolved expression of the *achaete* and *scute* genes in a position-specific manner (Gomez-Skarmeta et al., 2003; Modolell and Campuzano, 1998). Afterwards, the PNC gives rise to one or two sensory organ precursor (SOP) cells. Each SOP cell then differentiates into five cells, the bristle (macrochaete), socket, neuron, sheath, and glial cells, thereby forming mature mechanosensors.

Numerous molecular players involved in this process have been identified, including two dCtBP-interacting factors, Hairless (H) and U-shaped (Ush) (Bardin et al., 2004; Gomez-Skarmeta et al., 2003; Pi and Chien, 2007; Reeves and Posakony, 2005). The H adaptor protein promotes SOP fates by recruiting the dCtBP and Groucho corepressors, thus maintaining the default state of Notch target genes (Bang et al., 1991; Bang and Posakony, 1992; Barolo et al., 2002; Castro et al., 2005). Interestingly, phenotypic descriptions of a weak *dCtBP* mutation have documented duplicated thoracic bristles defects (Poortinga et al., 1998; Stern et al., 2007). However, *H* mutations induce loss of bristles (Bang et al., 1991; Bang and Posakony, 1992). These observations would be controversial if dCtBP acted only through H.

Ush, which contains a PxDLS dCtBP-interacting motif, regulates the expression of the *achaete* and *scute* genes through a dorsocentral (DC) enhancer element, thereby specifying the DC PNC (Cubadda et al., 1997; Garcia-Garcia et al., 1999; Gomez-Skarmeta et al., 2003; Haenlin et al., 1997). The vertebrate orthologs of Ush, Friend of GATA 1 (FOG-1) and FOG-2, have been shown to interact with CtBP through the PxDLS motifs *in vitro* and in co-immunoprecipitation assays (Fox et al., 1999; Holmes et al., 1999; Katz et al., 2002). Similarly, Ush binds to dCtBP *in vitro* (Waltzer et al., 2002). Expression of *ush* and *pannier* (*pnr*) is initiated by the Decapentaplegic morphogen gradient, but the *ush* expressing domain in the most dorsal region of the wing disc is slightly restricted compared to *pnr* expression (Fromental-Ramain et al., 2008; Garcia-Garcia et al., 1999; Sato and Saigo, 2000; Tomoyasu et al., 2000). In the absence of Ush, Pnr (GATA transcription factor) directly activates *achaete* and *scute* expression by GATA sites in the DC enhancer, Ush, however, represses expression by directly interacting with enhancer-bound Pnr (Garcia-Garcia et al., 1999). Similarly, FOG-1 and FOG-2 are also known to bind GATA-family transcription factors and function as corepressors (Deconinck et al., 2000; Fox et al., 1999; Holmes et al., 1999). *ush* loss-of-function mutants show extra DC and scutellar (SC) bristles on the fly notum, while overexpression of this corepressor causes a reduction of the DC bristles (Cubadda et al., 1997). To date, roles mediated by the dCtBP-Ush complex in this process are largely unknown.

In this study, we show that dCtBP is required for the proper patterning of the fly PNS. In the absence of *dCtBP* gene function, alterations in the mechanosensory bristle pattern were observed; in many cases ectopic or additional bristles formed, in agreement with the appearance of extra SOP cells. Towards a better understanding of how dCtBP functions to regulate this process, we identified *ush* and *pnr* as genes that were able to genetically interact with *dCtBP*. Disruption of the PxDLS motif in Ush partially impaired its function. In addition, one of the phenotypes (bald cuticle) seen in *dCtBP* mutants in this study was also observed in *H* mutants (Bang et al., 1991; Bang and Posakony, 1992). Furthermore, one particular phenotype induced by *dCtBP* mutations, the double bristle with sockets phenotype, suggests that additional molecular pathways involve dCtBP, independently of its interaction with both H and Ush. Taken together, we propose that dCtBP is involved in regulating different aspects of the gene network responsible for specifying the PNS *via* multiple mechanisms.

2. Results

2.1. *dCtBP is involved in mechanosensory organ formation*

Reduction of *dCtBP* activity results in a series of dorsal thoracic mechanosensory defects (Fig. 1). In this study, we used two *dCtBP* alleles: the hypomorphic $dCtBP^{03463}$ allele and the EMS-induced $dCtBP^{87De\text{-}10}$ mutation, the most severe mutant allele that is publicly available (Barolo et al., 2002; Poortinga et al., 1998; Stern et al., 2007). The most pervasive of these abnormalities are extra bristles (additional bristles attached to sockets) (Fig. 1C–E). Less frequently, double bristles (two bristles housed in the same socket structure) (Fig. 1E) and bald cuticle (complete loss of both bristles and their associated sockets) (Fig. 1F) are observed. Quantitative analysis of these bristle phenotypes shows that a subset of bristles, such as the posterior SC (PSC), anterior SC (ASC), posterior DC (PDC), anterior DC (ADC), posterior postalar (PPA), anterior PA (APA), and posterior supraalar (PSA) bristles, is affected in $dCtBP^{03463}/dCtBP^{03463}$ and $dCtBP^{87De\text{-}10}/dCtBP^{03463}$ pharate adults (Fig. 2). Other bristles, including the anterior SA (ASA), presutural (PS), posterior notopleural (PNP) and anterior NP (ANP) bristles, were relatively insensitive to reduction of *dCtBP* activity (Fig. 2). The ASC region most frequently displayed additional bristles. This correlates with the haplo-insufficiency observed for the ASC region in both