CHAPTER 6

CtBP Proteins in Vertebrate Development

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Abstract

The fundamental question facing developmental biology is how the diversity of cell and tissue types that comprise a vertebrate organism can be generated from a single fertilized egg. A critical aspect of the developmental process is setting up and maintaining the differential gene expression that is required to establish the variety of cell lineages present in the adult organism. Thus, an important aspect of understanding development is understanding how early asymmetries in the transcriptome of various cell types are established and, once established, how they are maintained or modified through subsequent generations and differentiation events. This process is carried out by the combined activities of both sequence specific DNA binding factors and their associated coactivators and corepressors that act on either the general transcriptional machinery or the histone component of chromatin. CtBP proteins comprise one branch of corepressors that get recruited to DNA via sequence-specific DNA binding proteins and regulate gene expression. In mice, the CtBP family proteins are encoded by two loci, *Ctbp1* and *Ctbp2*. The transcripts encoding the CtBP1 and CtBP2 proteins are widely expressed and exhibit both unique and shared expression domains in the developing embryo. Genetic analysis of mice harboring mutations in *Ctbp1* and *Ctbp2* indicate that the proteins they encode likely have redundant functions during embryogenesis but are differentially required for specific developmental processes. This analysis shows that CtBP proteins are important in the formation of the placenta and tissues derived from all three germ layers, including muscles, skin, neural ectoderm, and intestinal epithelium. This chapter focuses on the roles of CtBP1 and CtBP2 proteins in vertebrate development, with an emphasis on the genetics of *Ctbp1* and *Ctbp2*, the possible pathways that utilize CtBP proteins during embryogenesis, and the evidence that CtBP proteins could be implicated in multiple developmental processes linked to human diseases.

CtBP Expression in Vertebrate Development

In vertebrates, the *Ctbp* gene family is likely comprised of two loci, *Ctbp1* and *Ctbp2*, which appear to encode at least 4 protein isoforms. The *Ctbp2* locus encodes CtBP2 and Ribeye, a protein isoform with a different N-terminus that is encoded from the alternative inclusion of exons 5' to the start site of *Ctbp2*.[1,2] The *Ctbp1* locus appears to encode CtBP1 and CtBP3/BARS, again via differential expression of 5' exons.[3] In both mice and *Xenopus*, CtBP genes exhibit widespread expression patterns and, between the two genes, are likely expressed in all cell types of the developing embryo.[4-6] This is similar to what is seen in *Drosophila*, where *dCtbp* is maternally expressed and deposited in the embryo and is uniformly expressed in the embryo, albeit at lower levels, following the onset of zygotic gene

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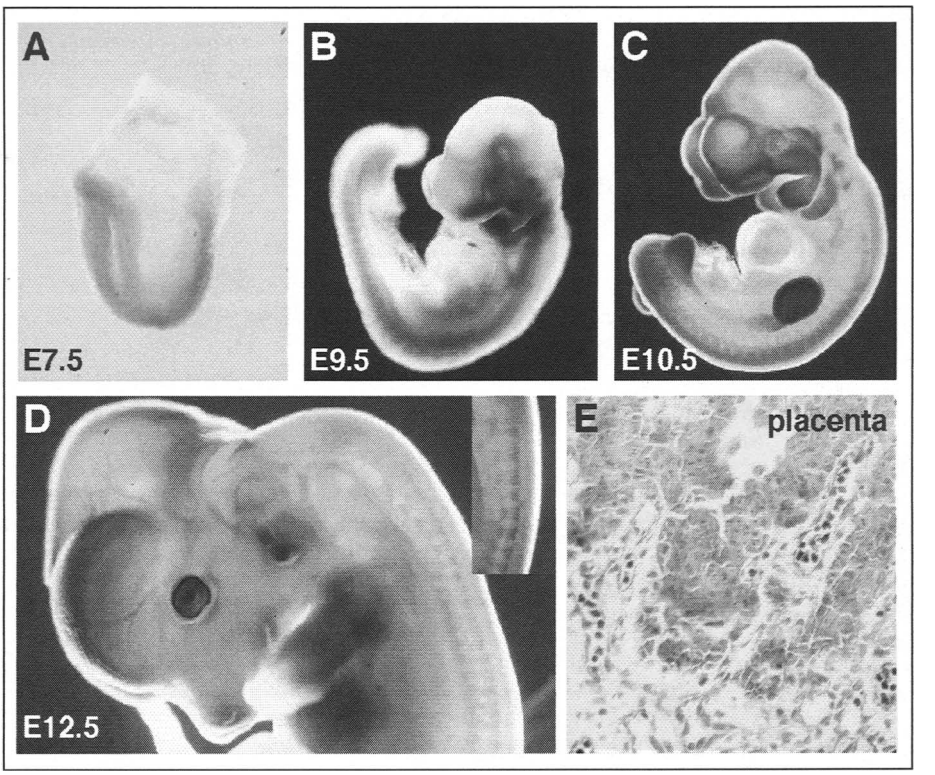

Figure 1. Expression of Ctbp2 during mouse development. A-D). Mouse embryos were isolated at the indicated developmental stages and stained to detect the activity of the *Ctbp2* promoter by staining embryos with X-gal to detect expression of the β-galactosidase reporter in the gene trap cassette integrated into the *Ctbp2* locus. In (A), anterior is to the left. Inset in (D) shows expression in the dorsal root ganglia. E) The placenta from an E.10.5 embryo heterozygous for the gene trap insertion into *Ctbp2* was isolated, stained with x-gal, and sectioned to demonstrate expression in the chorio-allantoic plate (cp) and vasculature of the labyrinth layer (lb).

expression.[7,8] Furthermore, the expression of *Ctbp2* is essentially uniform in the embryo at embryonic day (E) 7.0 and appears to be more dynamic as development progresses (Fig. 1). While the expression of *Ctbp1* and *Ctbp2* appear to be widespread, there are clear regions where these genes are more highly expressed. From approximately E7.0-E10.5 of development, *Ctbp2* expression is essentially uniform. However, by E12.5, regions of higher expression are seen in the developing nervous system, eye, and ear. In extraembryonic portions of the embryo, *Ctbp2* expression is restricted to the vasculature of the yolk sac, the chorio-allantoic plate, and the vasculature of the labyrinth layer of the placenta. This expression is consistent with the defects observed in *Ctbp2* mutant embryos (see below).

Like *Ctbp2, Ctbp1* is also widely expressed in the developing mouse embryo and shows highest expression in the neural epithelium.[4,5] The largely overlapping expression of these two genes in the embryo likely accounts for the lack of embryonic phenotypes observed in the individual knock-outs lines of mice. Consistent with this idea, is the observation that there is little detectable CtBP1 protein in the extra-embryonic structures that express CtBP2.