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Characterization of *Bcor* expression in mouse development

Joseph A. Wamstad [a], Vivian J. Bardwell [a,b,*]

[a] *Molecular, Cellular, Developmental Biology and Genetics Graduate Program, University of Minnesota, 6-160 Jackson Hall, 321 Church St SE, Minneapolis, MN 55455, USA*

[b] *Department of Genetics, Cell Biology and Development and Cancer Center, University of Minnesota, 6-160 Jackson Hall, 321 Church St SE, Minneapolis, MN 55455, USA*

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Abstract

Mutation of the gene encoding the transcriptional corepressor BCOR results in the X-linked disorder Oculofaciocardiodental syndrome (OFCD or MCOPS2). Female OFCD patients suffer from severe ocular, craniofacial, cardiac, and digital developmental defects and males do not survive through gestation. BCOR can mediate transcriptional repression by the oncoprotein BCL6 and has the ability to reduce transcriptional activation by AF9, a known mixed-lineage leukemia (MLL) fusion partner. The essential role of BCOR in development and its ability to modulate activity of known oncogenic proteins prompted us to determine the expression profile of *Bcor* during mouse development. Identification of independently transcribed exons in the 5′ untranslated region of *Bcor* suggests that three independent promoters control the expression of *Bcor* in mice. Although *Bcor* is widely expressed in adult mouse tissues, analysis of known spliced isoforms in the coding region of *Bcor* reveals differential isoform usage. Whole mount in situ hybridization of mouse embryos shows that *Bcor* is strongly expressed in the extraembryonic tissue during gastrulation and expression significantly increases throughout the embryo after embryonic turning. During organogenesis and fetal stages *Bcor* is differentially expressed in multiple tissue lineages, with a notable presence in the developing nervous system. Strikingly, we observed that *Bcor* expression in the eye, brain, neural tube, and branchial arches correlates with tissues affected in OFCD patients.

Keywords: *Bcor*; Oculofaciocardiodental syndrome; MCOPS2; Extraembryonic; BCL6; OFCD; Lenz; PcG; AF9

1. Results and discussion

BCOR (BCL6 corepressor) is a transcriptional corepressor that was identified based on its ability to interact with the POZ domain of the oncoprotein BCL6 (Huynh et al., 2000). Chromosomal translocations in the promoter and 5′ untranslated region of the human BCL6 gene are a common genomic alteration in non-Hodgkin's B cell lymphomas (Baron et al., 1993; Dalla-Favera et al., 1999; Kerckaert et al., 1993; Miki et al., 1994; Ye et al., 1993). These translocations result in aberrant expression of BCL6 (Chen et al., 1998; Ye et al., 1995). Mice engineered to model one of these translocations develop B cell lymphomas, demonstrating that BCL6 is a bona fide oncogene (Cattoretti et al., 2005). BCOR potentiates BCL6 mediated transcriptional repression of reporter constructs in transiently transfected cells (Huynh et al., 2000). In germinal center B cells, BCOR is found with BCL6 at several known BCL6 target genes, including regulators of cell proliferation and apoptosis (Gearhart et al., 2006). BCOR co-purifies with an 800 kDa complex comprised of Polycomb group transcriptional repressor proteins and SCF ubiquitin E3 ligase components (Gearhart et al., 2006). Epigenetic modification of BCL6 target gene chromatin by the BCOR repression complex is likely to play a role in mediating their repression.

In addition to its role in B-cells, BCOR aids in the control of gene expression in multiple tissues and organ systems during development and into adulthood as mutations in human

* Corresponding author. Address: Department of Genetics, Cell Biology and Development, and Cancer Center, University of Minnesota, 6-160 Jackson Hall, 321 Church St SE, Minneapolis, MN 55455, USA. Tel.: +1 612 626 7028; fax: +1 612 626 7031.
E-mail address: bardw001@umn.edu (V.J. Bardwell).

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BCOR result in X-linked Oculofaciocardiodental syndrome (OFCD) (Ng et al., 2004). OFCD is the primary subtype of OMIM #300166 microphthalmia, syndromic 2 (MCOPS2) and is characterized by ocular, dental, cardiac and digital anomalies in heterozygous females (Ng et al., 2004; Schulze et al., 1999). Males with OFCD do not survive due to presumed embryonic lethality (Ng et al., 2004). Since *BCOR* lies on the X chromosome in both mice and humans, random X-inactivation results in mosaic expression of the mutant allele contributing to varying disease severity in females (Ng et al., 2004; Schulze et al., 1999). Additionally, peripheral leukocytes of female patients show preferential survival of cells in which the mutant allele of *BCOR* lies on the inactivated X chromosome, indicating a strong requirement for *BCOR* in hematopoiesis (Hedera and Gorski, 2003; Ng et al., 2004). Another form of MCOPS2, which is distinct from OFCD, occurs in males with a single missense mutation (p.P85L) in the fourth coding exon of *BCOR* (Horn et al., 2005; Ng et al., 2004). In the described family, this syndrome is inherited in an X-linked recessive pattern and comprises microphthalmia/anophthalmia, mental retardation, and skeletal and other anomalies (Ng et al., 2002). RNAi knock-down of *Bcor* in zebrafish results in eye, skeletal, and nervous system abnormalities consistent with those found in MCOPS2 patients (Ng et al., 2004). BCOR has also been shown to interact with and repress transcriptional activation of the MLL fusion protein AF9 (Srinivasan et al., 2003), a known regulator of Hox gene expression (Collins et al., 2002) and skeletal development, further implicating BCOR as a key developmental regulator. The pleiotropic effects induced by the loss of functional BCOR in humans and zebrafish (Ng et al., 2004) clearly illustrate the essential role of *BCOR* during embryogenesis and emphasizes the importance of determining the spatial and temporal expression of *BCOR* during development. Herein, we provide a detailed analysis of *Bcor* mRNA expression during mouse development and in the adult mouse.

In adult mice and humans, *Bcor* is widely expressed (Huynh et al., 2000; Nagase et al., 2000). However, these studies are limited in scope, relying on RNA dot blot analysis of human tissues and reverse transcription PCR (RT-PCR) on a limited number of mouse tissues. Expanding upon these results, we have dissected the pattern of *Bcor* expression by Northern blot analysis, more extensive RT-PCR, and whole mount and section in situ hybridization experiments. To determine the expression pattern of *Bcor* in adult mouse tissues, we harvested total RNA from 14 different organs and conducted Northern blot analysis on 4 organs and RT-PCR on all 14 organs using a probe that recognizes all mRNA isoforms (Fig. 1F). Three transcripts migrating at approximately 7 kb are found in all four organs analyzed, however, the stoichiometry of the different transcripts is not identical (Fig. 1A). *Bcor* mRNA is detected by RT-PCR in all tissues tested (Fig. 1B). The ubiquitous expression of *Bcor* in adult mouse tissue is consistent with expression of *BCOR* in human adult tissue (Huynh et al., 2000).

The identification of three *Bcor* transcripts by Northern blot analysis prompted us to consider that the *Bcor* gene may utilize multiple promoters, alternative splicing and/or polyadenylation sites. Although alternative splicing in the coding region has been reported (Fig. 1F and Srinivasan et al., 2003) this can only affect transcript size by up to 156 bp. In silico analysis of spliced expressed sequence tags (EST) databases supported use of alternative promoters and polyadenylation sites at the mouse *Bcor* genomic locus. Two alternative polyadenylation sites separated by 550 bp are present. CpG islands are also found in close proximity upstream of exons 1a, 1b, and 1c, further supporting the presence of three independent promoters (Fig. 1F). To determine whether all three promoters suggested by the EST database are actively used in most tissues, we generated an exon 2 specific reverse primer and three forward primers in putative *Bcor* exons 1a, 1b, and 1c that correspond with sequences specifically driven by putative promoters P1, P2, and P3 (Fig. 1F). RT-PCR and sequencing of the products demonstrates that *Bcor* uses all three putative independent promoters (Fig. 1B and data not shown). In the panel of adult mouse tissues tested, each promoter appears to be used at similar levels in all tissues tested, with the exception of whole blood, which appears to preferentially use promoter 3 relative to other tissues. Interestingly, amplification from promoter 2 results in two specific amplicons (Fig. 1B) due to the use of an alternative splice donor sites in exon 1b (data not shown). We also examined promoter use during embryonic development (E11.5–E18.5). Although all promoters are used at these stages the distal splice donor of exon 1b is not used at E15.5 and E16.5 (Fig. 1D).

Splicing bypassing exon 5 and/or alternative splice acceptor usage at exon 8 results in the previously identified *Bcor* isoforms a–d (Fig. 1F) (Srinivasan et al., 2003). Only isoforms a and b contain sequences required for the interaction with the transcriptional regulator AF9 (Ng et al., 2004; Srinivasan et al., 2003). To determine which isoforms are differentially expressed in the embryo and adult mouse tissues, we conducted RT-PCR using primers that span exon 4 to exon 10 on samples used in Fig. 1B and D. *Bcor* isoforms a, c, and d can be amplified from the samples tested (Fig. 1C and E). Isoform c shows the most ubiquitous expression in the sample set and isoform a shows the most tissue-specific expression. Relative to the other tissues, isoform a is more strongly represented in the brain and testis. Isoform b is only barely detected in ovary, eye, spleen, and kidney at this level of amplification but not at embryonic stages.

The clinical presentation of OFCD in female patients and presumed embryonic lethality in males suggested that *Bcor* might have a unique expression pattern during embryogenesis that might give insight into future studies on *Bcor* function. To determine the spatial and temporal expression of *Bcor* during gastrulation and early organogenesis we conducted whole mount and section in situ hybridization on embryonic day 7.5–15.5 CD-1 outbred