

Gene expression pattern

# Identification and expression of the mammalian homologue of *Bicaudal-C*

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## Abstract

Translational activation and repression play an important role in the spatial-temporal regulation of gene expression in embryonic development. Bicaudal-C is an RNA-binding molecule believed to function at this post-transcriptional level. Loss-of-function mutants in *Drosophila* affect anterior-posterior patterning because of ectopic and premature translation of the posterior determinant *oskar*. The *Xenopus* homologue of Bicaudal-C is one of the few molecules that, when microinjected ectopically, results in endoderm formation in the absence of mesoderm induction. Here we report the sequence and expression pattern of the murine and human homologues of *Bicaudal-C*. The human gene is located on chromosome 10q21.2. Expression analysis in mouse using in situ hybridization detects expression of *Bicaudal-C* not only in domains detected in *Xenopus*, but also in previously unreported regions. As in *Xenopus*, mouse *Bicaudal-C* mRNA is found in the growing oocyte, Hensen's node, and the developing kidney. Additionally, at later stages it is strongly expressed in the developing gut endoderm, in areas of cartilage formation, in pleuro-peritoneal membrane derivatives, in lung mesenchyme, and in the stroma of the ovary. We conclude that mouse *Bicaudal-C* is a maternally provided gene product that is tightly regulated during mammalian cell differentiation. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Bicaudal-C; Cartilage primordia; Diaphragm; Endoderm; KH domain; Lung; Mouse; Node; Mesonephros; Metanephros; Oocyte; Ovary; Pericardium; Pleuro-peritoneal membrane; mRNA; RNA binding; SAM; Translational regulation

## 1. Results

A *Xenopus* homologue of the *Drosophila* maternal effect gene Bicaudal-C has recently been described (Wessely and De Robertis, 2000). Using sequence information deposited in the publicly available databases we identified a cDNA clone (GenBank AW240366) encoding the full length mRNA of murine *Bicaudal-C* (*mBic-C*). At the same time, high throughput genomic sequences from the Human Genome Program revealed genomic clones encoding the human version of the protein (GenBank AC009933 and AC012168). These mapped to chromosome 10q21.2, a locus that has been implicated in at least four human diseases (<http://www.ncbi.nlm.nih.gov:80/htbin-post/Omim/getmap?chromosome=10q21.1>). Using the sequence information from the mouse and the *Xenopus* clone as well as software predicting the localization of splice donor and acceptor sites, we assembled the putative sequence of the human Bicaudal-C. *hBic-C* is encoded by 21 exons spanning a region of at least 72 kb (Fig. 1A). Both the mouse and

the human Bic-C proteins present the overall domain structure of the *Xenopus* and *Drosophila* proteins (Fig. 1B,C; Mahone et al., 1995; Wessely and De Robertis, 2000): an N-terminal RNA binding domain consisting of five KH domains (Adinolfi et al., 1999) and a C-terminal SAM domain thought to be involved in protein-protein interaction (Schultz et al., 1997). Mouse and human Bic-C are 78.6% and 81.3% identical, respectively, when compared to the *Xenopus* homologue, and 89.8% when compared among themselves (Fig. 1D).

In the adult mouse, Northern blot analysis showed that *mBic-C* is predominantly expressed in heart and kidney. Low levels of message were observed in testis, while brain, spleen, lung, liver, and skeletal muscle were devoid of detectable *mBic-C* expression (Fig. 1E).

Since *xBic-C* mRNA can first be detected in the maturing *Xenopus* oocytes (Wessely and De Robertis, 2000), cryostat sections from ovaries of 8 week old mice were analyzed by non-radioactive in situ analysis. As seen in Fig. 2A–C, *mBic-C* mRNA is present in growing primary oocytes and in the stromal cells of the theca, but only at lower levels in the granulosa cells surrounding the oocyte. Closer examination of the staining in the oocytes suggested that some might be associated with oocyte cytoplasmic organelles, perhaps

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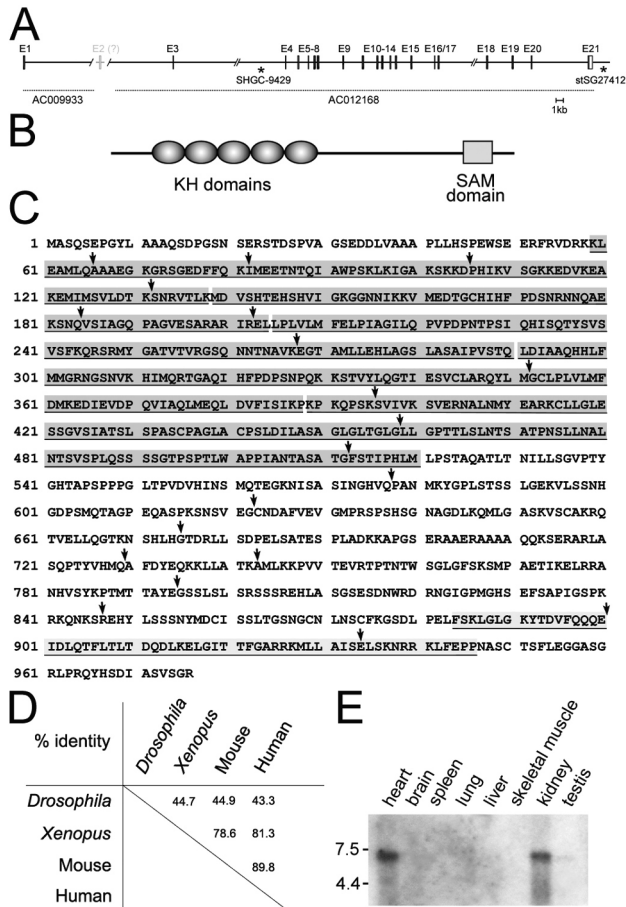


Fig. 1. Sequence analysis of mouse and human Bicaudal-C. (A) Genomic exon/intron structure of the human *Bic-C* locus. Filled boxes indicate the exons encoding the protein, while the open box represents the 3' untranslated region. Two genomic BAC clones (GenBank AC009933 and AC012168) are indicated by a dotted line. Exon 2 could not be identified in the publicly available sequences and therefore is still speculative. The gene is located on chromosome 10q21.2 and the position of two STS markers (SHGC-9429 and stSG27412) are indicated by asterisks. (B) Schematic representation of the Bicaudal-C protein containing a N-terminal RNA binding domain composed of 5 KH RNA-binding motifs and a C-terminal SAM domain thought to be an interface for both homo- and heterodimerization. (C) Protein sequence of *mBic-C*. The five KH domains and the SAM domain are outlined by gray boxes. The arrows indicate the position of the exon/intron borders of the human genomic locus. (D) Sequence comparison between the *Drosophila* (GenBank S55051), the *Xenopus* (GenBank AF224746), the mouse and the human Bicaudal-C proteins. (E) Northern blot of adult murine tissues hybridized with a probe for *mBic-C*. Note the strong expression of the RNA in heart and kidney. The lanes are about equally loaded (see Pappano et al., 1998 for normalization with  $\beta$ -actin).

corresponding to the Golgi complex (Fig. 2B). Primordial follicles, which have not yet started their growth process, also contain cytoplasmic *mBic-C* transcripts (data not shown). We conclude that despite the very different modes of development in *Xenopus* and mouse, *Bic-C* transcripts are provided maternally.

After fertilization and implantation, expression of *mBic-*

*C* can be first detected by whole-mount in situ hybridization at the rostral tip of the primitive streak, Hensen's node, at the late streak stage (Fig. 2E). At the late headfold stage *mBic-C* expression specifically demarcates the layer of the node from which definitive endoderm and midline mesoderm arises (Fig. 2G,H). Interestingly, in contrast to many other genes expressed in the node at this stage, *mBic-C* is never detected in the primitive streak (Fig. 2D–F) suggesting that it marks the undifferentiated cells of this organizing center. At 6–8 somite stage *mBic-C* is observed in the definitive endoderm (Fig. 2I,J). Strong expression can be detected in the caudal intestinal portal (Fig. 2K,L). At 12–15 somite stage *mBic-C* is still present in the hindgut, but transient expression can also be seen in tissues of neural and mesodermal origins (Fig. 2M).

At E13 in situ hybridizations on sections detected novel sites of *mBic-C* expression, not previously reported for *Xenopus*. At this stage *mBic-C* mRNA is present around all sites of cartilage formation, such as cervical vertebral bodies, ribs, and Merckel's cartilage (Fig. 3A–D). Additionally, *mBic-C* is expressed in the derivatives of the pleuro-peritoneal membrane, the diaphragm and the pericardium (Fig. 3A), as well as the mesenchyme of the developing lung (Fig. 3E). Finally, while in *Xenopus* *xBic-C* was only reported in the pronephros, a kidney structure only present in higher vertebrates as a rudiment (Goodrich, 1930), in mouse it is expressed in both the mesonephros and metanephros (Fig. 3F,G).

In summary, *mBic-C* encodes a potential translational regulator whose expression correlates with undifferentiated tissues such as oocytes, the node and developing mesenchyme condensations.

## 2. Experimental procedures

The GenBank accession number for *mBic-C* is AF319464. Northern blot analysis was performed using a mouse multiple tissue Northern blot (Clontech) and an *EcoRI/NotI* fragment of the *mBic-C* cDNA labeled by random priming (Stratagene). For the synthesis of digoxigenin labeled anti-sense probe the full-length *mBic-C* EST was linearized with *Sall* and transcribed using the T7 RNA polymerase. In situ hybridizations were performed as previously described (Belo et al., 1997; Henrique et al., 1995). Embryos were staged according to Downs and Davies (1993).

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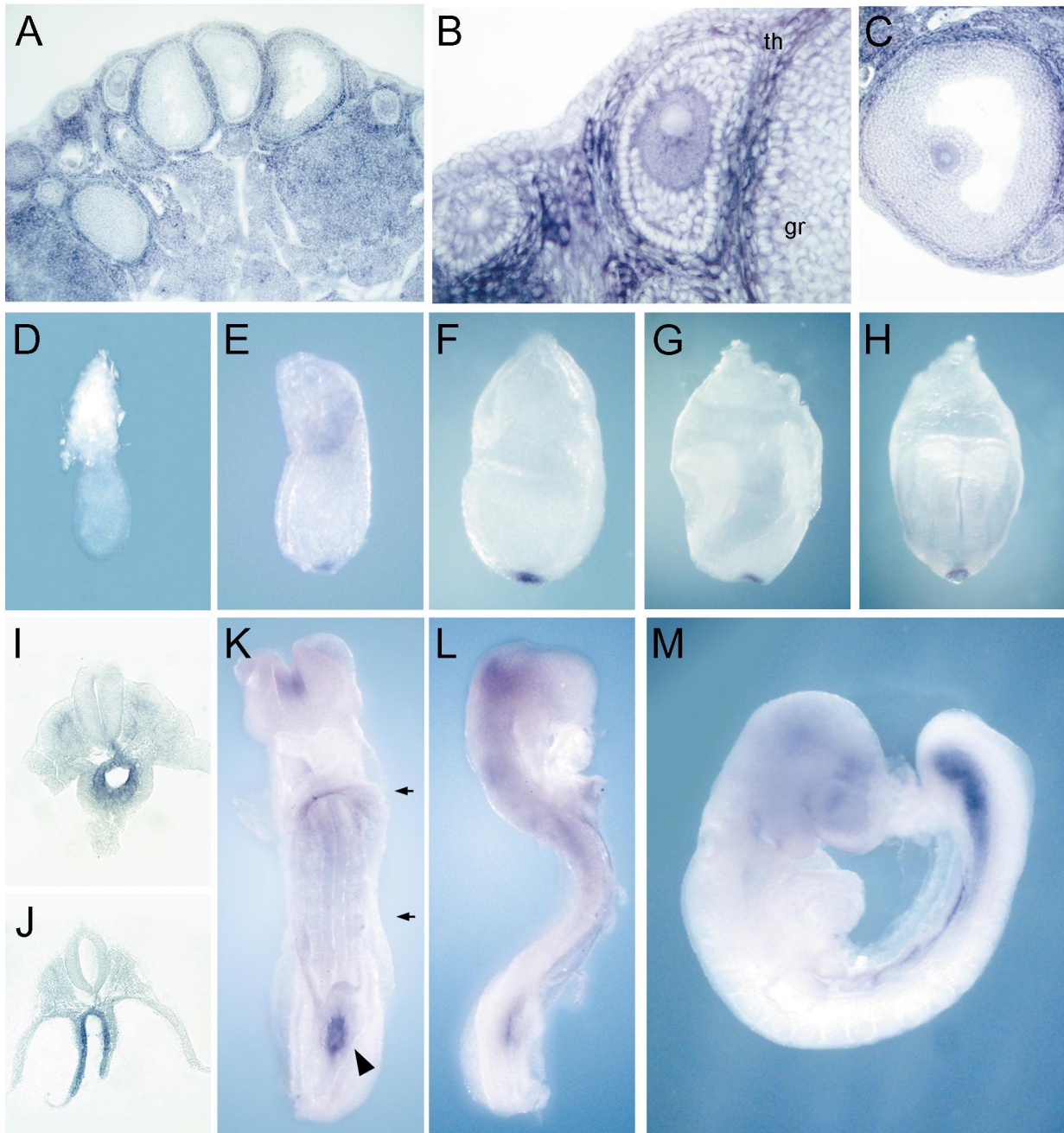


Fig. 2. Expression of *mBic-C* in ovaries and embryos from pre-streak through 12–15 somite stages. (A–C) Non-radioactive in situ hybridization on sections through ovaries of 8-week mice predominantly detects *mBic-C* transcripts in the growing primary oocyte, in the theca interna and at lower levels in the stratum granulosum. (B) Close up of a primary follicle. (C) Vesicular follicle of Graaf, note *mBic-C* signal in the oocyte. (D–H) Expression of *mBic-C* in the node of the developing embryo. Lateral views of pre-streak (D), late streak (E), late bud neural plate (F), and late headfold stages (G) as well as anterior view of late headfold stage (H). Note that expression of *mBic-C* is specifically restricted to the node and is never detected in the primitive streak. (I,J) Transverse sections, (K) ventral and (L) lateral views of 6–8 somite stage embryos. The levels of the sections are indicated by arrows in (K). Arrowhead in (K) marks the caudal intestinal portal. (M) At 12–15 somite stage the expression pattern of *mBic-C* becomes more complex. In addition to expression in the hindgut, expression can at this stage be detected in tissues of mesodermal and neural origin.

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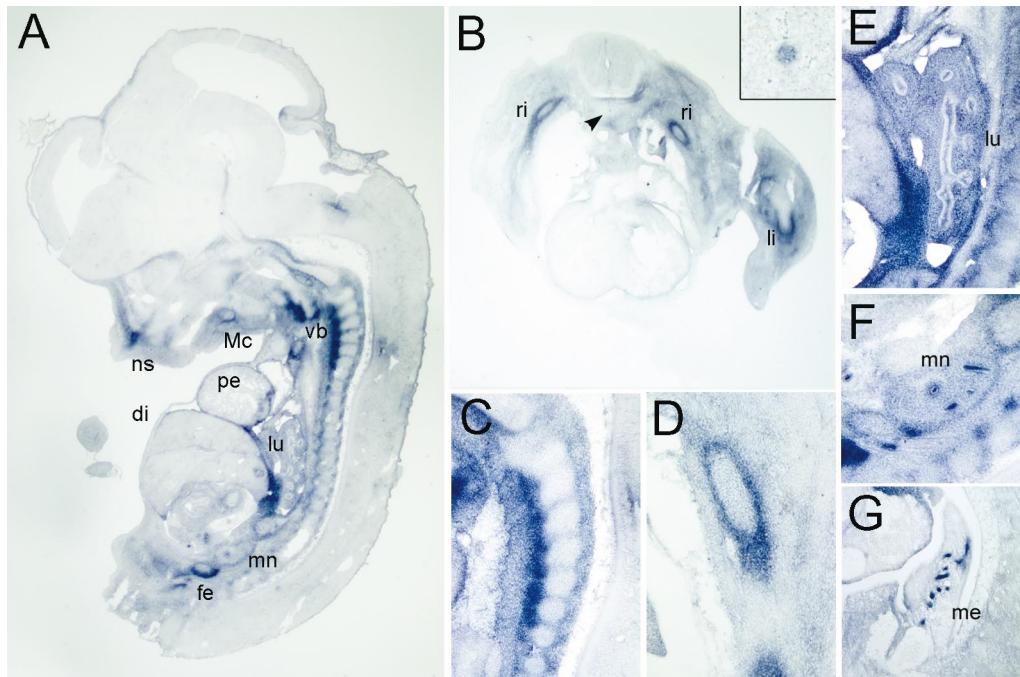


Fig. 3. Expression of mouse Bicaudal-C at E13 by in situ hybridization on sections. (A) Sagittal and (B) transverse sections show expression of *mBic-C* around a variety of cartilage primordia such as the nasal septum (ns), Meckel's cartilage (Mc), the head of the femur (fe), the vertebral bodies (vb), the ribs (ri) and in the limbs (li). Additionally, *mBic-C* mRNA can also be detected in the diaphragm (di), the pericardium (pe), the metanephros (mn), the mesenchyme of the lung (lu) and the notochord (arrowhead and inset in B). (C–F) Higher magnification of the expression of *mBic-C* at E13 in the vertebral bodies (C), the ribs (D), the lung (E) and the metanephros (F). (G) Staining in the mesonephros (me) at E10.

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