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Gene expression pattern

RanBP1, a velocardiofacial/DiGeorge syndrome candidate gene, is expressed at sites of mesenchymal/epithelial induction

Thomas M. Maynard[a], Gloria T. Haskell[a], Naina Bhasin[a], James M. Lee[a], Andrew A. Gassman[a], Jeffrey A. Lieberman[b], Anthony-Samuel LaMantia[a,*]

[a]*Department of Cell and Molecular Physiology and UNC Neuroscience Center, CB#7545, School of Medicine, University of North Carolina, Chapel Hill, NC, 27599, USA*

[b]*Department of Psychiatry, School of Medicine, University of North Carolina, Chapel Hill, NC, USA*

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Abstract

RanBP1, a velocardiofacial syndrome/DiGeorge syndrome candidate gene, is expressed in the frontonasal processes, branchial arches, aortic arches, and limb buds. At these sites, *RanBP1* apparently coincides with neural crest-derived mesenchymal cells. In addition, *RanBP1* is expressed in the forebrain as well as in hindbrain regions previously associated with crest-derived mesenchymal cells.

Keywords: *RanBP1*; Velocardiofacial syndrome; DiGeorge syndrome

1. Results

RanBP1 (also referred to as Htf9a) was originally identified as a transcription product of a randomly isolated CpG island (Lavia et al., 1987; Bressan et al., 1991). *RanBP1* is a binding partner of the Ras-related nuclear protein Ran/TC4, and regulated in a cell cycle-dependent manner (Battistoni et al., 1997; Kalab et al., 1999; Guarguaglini et al., 2000). The human *RanBP1* gene is found within a region of chromosome 22q11.1 commonly deleted in velocardiofacial syndrome (VCFS; OMIM 192430) and DiGeorge syndrome (DGS; OMIM 188400). Most human 22q11 genes have mouse homologues, many of which are collinear in a syntenic region of chromosome 16 (Sutherland et al., 1998). The expression of some homologues – including *Tbx1* (Chapman et al., 1996; Chieffo et al., 1997), *Ufd1* (Yamagishi et al., 1999), *HIRA* (Wilming et al., 1997), and *DGCR2*/*Sez-12* (Taylor et al., 1997) – coincides with neural crest-derived mesenchymal cells which participate in inductive interactions (see LaMantia et al., 2000; Scambler, 2000).

RanBP1 is detected at high levels in the E10.5 embryo, as are other VCFS/DGS genes (Fig. 1). More specifically, *RanBP1* is detected at levels similar to other 22q11 homologues in cDNA samples from microdissected frontonasal mass, branchial arches, limbs, and heart, sites where crest-derived mesenchymal cells are concentrated. Whole-mount in situ hybridization on E10.5 embryos clarified *RanBP1* localization (Fig. 2A). Sense probes did not display significant background labeling, nor was there trapping of probe in central nervous system (CNS) ventricles or other embryonic lumens (Fig. 2B). *RanBP1* localization is similar to that of *DGCR2* (Taylor et al., 1997; Fig. 2C), another 22q11 homologue associated with crest-derived mesenchyme. In the head (Fig. 2E), *RanBP1* is expressed in the frontonasal processes (Fig. 2F), branchial arches (Fig. 2G), and limited domains within or adjacent to the hindbrain (Fig. 2H). In the trunk, *RanBP1* is expressed in the aortic arches (Fig. 2I) and limb buds (Fig. 2J). *RanBP1* is also expressed along the caudal neural tube (Fig. 2A).

To resolve *RanBP1* cellular localization, we examined sections of hybridized embryos. *RanBP1* is found in the mesenchyme of the lateral frontonasal process (Fig. 2K), branchial arches (Fig. 2L–N), and limb buds (Fig. 2O). *RanBP1* is also seen in the mesenchyme and epithelium of the aortic arch (Fig. 2P) and otic placode (Fig. 2Q). *RanBP1* is also evident in mesenchymal cells along the rhombic lip (Fig. 2R) and caudal hindbrain (Fig. 2S) – spatially coincident with crest-derived mesenchymal cells (Nichols, 1981; Serbedzija et al., 1992). *RanBP1* expressing cells are found along the forebrain ventricular surface (e.g. Fig. 2K); however, this expression is not seen along the entire neural tube. Finally, as early as E9.5, *RanBP1* is found in the

* Corresponding author.

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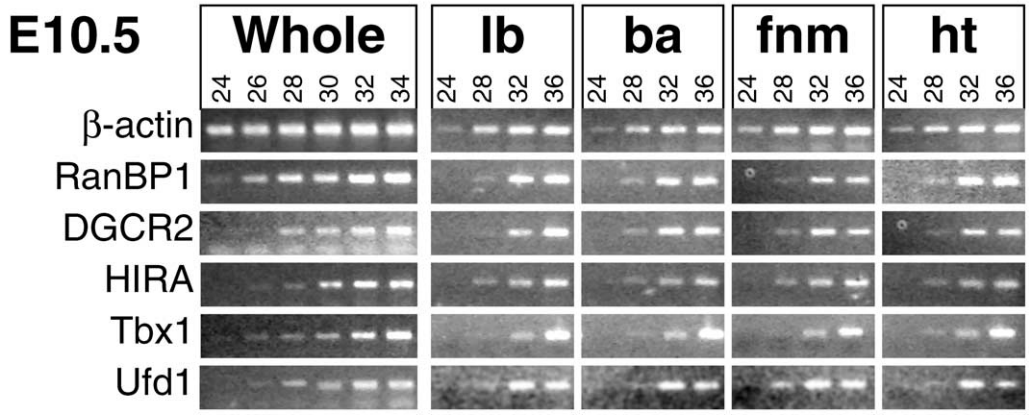

Fig. 1. Reverse transcriptase- (RT)- PCR based expression profile of *RanBP1* and other 22q11 homologues. Standardized cDNA pools were generated from either whole embryo RNA (left column) or from microdissected limb bud, branchial arch, frontonasal mass, or heart tissues (four right columns). The abundance of each gene was measured by examining the amplicon at sequential PCR cycles, as indicated. *RanBP1* was found at relatively high levels, as indicated by its appearance at early PCR cycles in both whole embryo and microdissected tissue cDNA pools. Its relative levels appear comparable or greater to the levels of the four other 22q11 gene homologues reported to be expressed at the sites of mesenchymal–epithelial inductive signaling, *DGCR2*, *HIRA*, *Tbx1*, and *Ufd1*.

frontonasal processes and the branchial arches (Fig. 2T), consistent with crest-derived mesenchymal populations observed by this age (Nichols, 1981; Serbedzija et al., 1992; Osumi-Yamashita et al., 1994). In addition, *RanBP1* is found along the dorsal aspect of the trunk neural tube and within the trunk mesoderm, coincident with nascent or migrating crest cells (Fig. 2U, V). Thus, *RanBP1* expression is primarily coincident with crest-derived mesenchymal cells, particularly at the sites of mesenchymal/epithelial inductive signaling.

2. Materials and methods

Embryos were obtained from a breeding colony of ICR (Harlan) mice maintained by the Department of Laboratory Animal Medicine at UNC, Chapel Hill, NC, USA. RNA was extracted with Trizol (Gibco) from E10.5 embryos or microdissected samples, and reverse transcribed to cDNA using random hexamer primers. The cDNA was standardized by determining relative dilutions that amplified beta-actin (using primers 5′-gatatcgctgcgctggtcgtc-3′ and 5′-tggcgtgagggagagcatagc-3′) to the same level as a competitive probe containing an engineered *Bam*HI site. cDNA pools were then assessed for the relative levels of each transcript by polymerase chain reaction (PCR) with primers for *RanBP1* (5′-gacccccagttcgagccaatagtt-3′ and 5′-ttccagcttctcggccacctttttc-3′), *DGCR2* (5′-catcctctcgctgctgcttttcat-3′ and 5′-ccccctggcggtgcttctgta-3′), *HIRA* (5′-aataattctggcccccactgctca-3′ and 5′-ctgctgttgctgtctccgctggta-3′), *Tbx1* (5′-accgcacccccaacctatgaaga-3′ and 5′-gcgggctggccaagtccta-3′), *Ufd1* (5′-caactcagccggctcaacattacc-3′ and 5′-agaaccagagaaggcacggaagc-3′), and beta-actin. All primers span at least one intron/exon boundary.

The 483 base *RanBP1* amplicon and the 537 base *DGCR2* amplicon were cloned into pBluescript II, and digoxigenin labeled probes (Roche) were made using T3 and T7 RNA polymerase (Promega). In situ hybidizations were performed as described previously (LaMantia et al., 2000). Stained and cleared embryos were digitally photographed; some were examined further after microdissection or cryosectioning.

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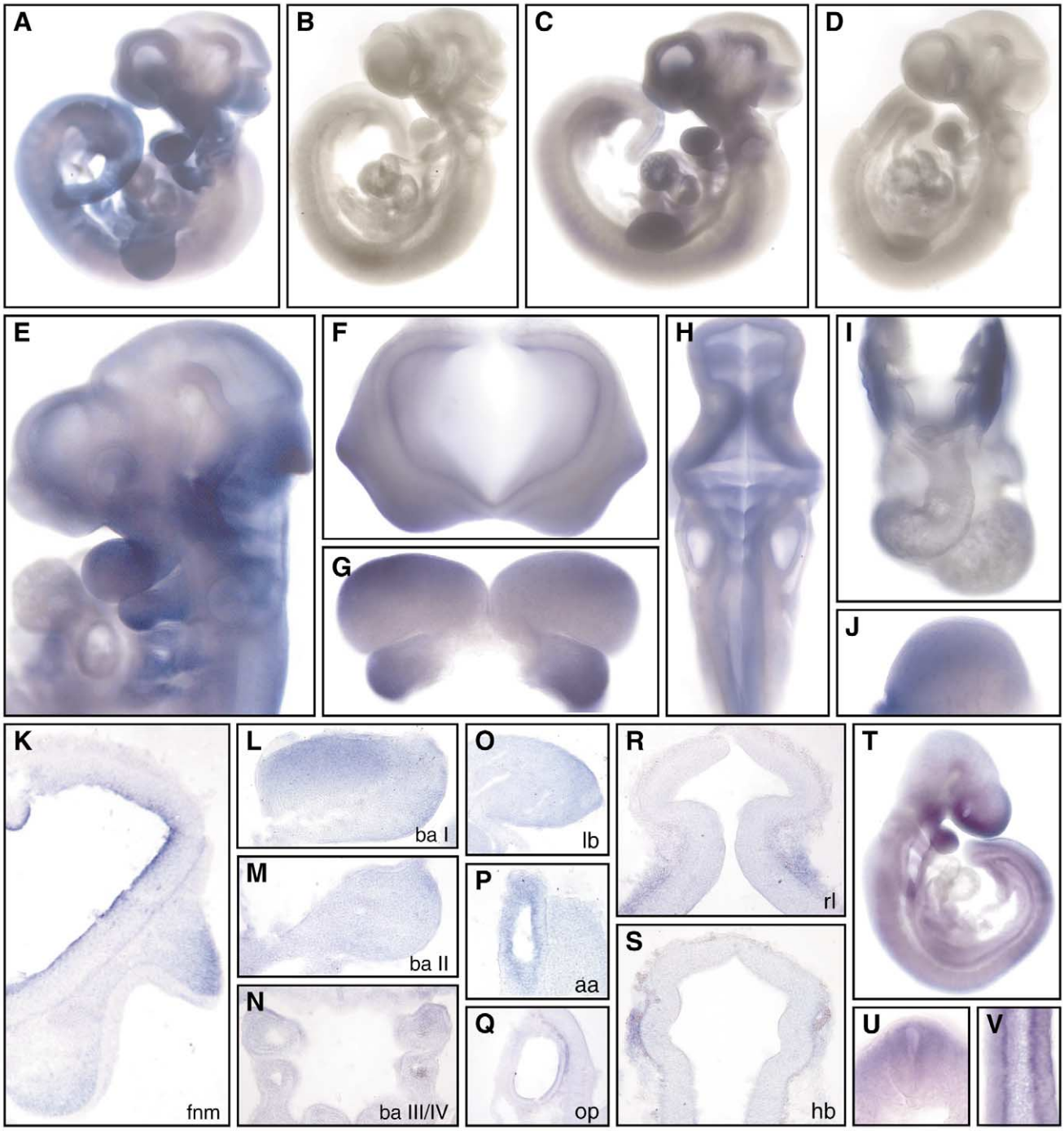

Fig. 2. Whole-mount RNA in situ hybridization analysis of gene expression. Hybridization of an E10.5 mouse embryo with antisense probe to *RanBP1* (A) and a sense control probe (B). The overall pattern of expression is similar to that observed in embryos hybridized with a *DGCR2* antisense probe (C); compare with respective sense control (D). In the head (E), *RanBP1* is expressed in the mesenchyme of the lateral frontonasal processes (F), the branchial arches (G), and in localized regions around the hindbrain (H). A concentrated stripe of *RanBP1* is also observed along the forming outflow tracts of the heart (I), and diffuse expression is seen within the limb bud (J). Cryosections (25 μm) of whole-mount stained embryos show *RanBP1* expression in the lateral frontonasal process (K), within the branchial arches (L–N) and the limb bud (O), and within the mesenchyme and epithelia of the aortic arch (P) and otic placode (Q). Message is also observed within mesenchymal cells along the developing hindbrain, as seen at the level of the rhombic lip (R) and at more caudal levels (S). At E9.5, *RanBP1* is also expressed at high levels within the frontonasal processes and branchial arches (T), as well as in the dorsal aspect of the caudal neural tube (U). This labeling corresponds to two dorsomedial stripes along the length of the caudal neural tube (V).

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