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EXPRESSION NOTE

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# Highly restricted *BMP10* expression in the trabeculating myocardium of the chick embryo

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**Abstract** Bone morphogenetic proteins (BMPs) control the development of diverse tissues during embryogenesis. Here, we report the expression of the *BMP10* gene during chick development. *BMP10* transcription is confined to the myocardium of atriae and ventricles in the forming heart from day 2 of development onwards (stage HH13). It correlates with the thickening of the innermost layer of the walls, i.e. the formation of myocardial ridges or trabeculae.

**Keywords** Heart · Bone morphogenetic protein · Ventricle · Atrium · TGF$\beta$

The first period of heart morphogenesis in the chick is the formation of a straight tube from the lateral mesoderm during the first 30 h of embryogenesis. Subsequently, the tube loops towards the right side, extracardial cells are recruited, and the morphogenesis of four primitive subdivisions, the inflow tract, the primitive atrium, the primitive ventricle and the outflow tract, is initiated (Larsen 1993). At the inflow side, part of the sinus venosus becomes incorporated into the developing atriae, whereas the rest of the atriae becomes diminished to the auricles. Only the myocardium of the auricles begins to thicken and myocardial ridges, the trabeculae, appear on the inner wall. At the outflow side of the heart the conotruncus, later the truncus arteriosus and the conus cordis, develops from an extracardial source of mesodermal cells, the secondary heartfield (Kelly et al. 2001; Mjaatvedt et al. 2001; Waldo et al. 2001). No trabeculation occurs in the outflow tract. The two ventricles are derivatives of the primary, paired heartfield. When their morphogenesis proceeds, the myocardium thickens and trabeculae appear on the inner wall, including also the anterior portion of the muscular ventricular septum. The trabeculated myocardium is the essential, functional component of the contractile heart tube. Here, we report the cloning of a trabeculation specific gene, the chicken ortholog of the previously isolated murine *BMP-10* gene (Neuhaus et al. 1999).

A chicken liver Lambda ZAP-cDNA library (Stratagene) was screened under low stringency conditions with a 379-bp XbaI/PstI fragment from the 3′end of the mouse BMP-9 ORF (Song et al. 1995). We obtained a cDNA of 2,374 bp, which contains an open reading frame of 1,149 bp followed by a 1,225-bp 3′UTR with a polyA tail. A translation start site is missing as indicated by a comparison of the chick cDNA with the closely related mouse cDNAs and the consensus sequences (Kozak 1984). The sequence is deposited in the EMBL database with the accession number AJ581667. The deduced amino acid sequence shows the characteristics of a member of the BMP family. The N-terminal propeptide region is followed by the C-terminal mature region with conserved seven cysteines. Both regions are separated by a proteolytic cleavage site (RIRR; Ozkaynak et al. 1992). Sequence comparisons reveal 77% amino acid identity to the murine BMP-10 protein, and 47% or 45% identity to the related chick dorsalin and mouse BMP-9 proteins, respectively. Therefore, we conclude to have cloned the orthologous chicken *BMP10* gene.

The embryonic expression was analyzed by whole-mount in situ hybridization in chick embryos. The first transcripts can be detected at HH13 (Hamburger and Hamilton stage 13) in the ventrolateral wall of the heart tube (Fig. 1A, B). This area corresponds to the future outer curvature, where the primitive ventricle will form. At HH15, the primitive myocardium has become multilayered. *BMP10* expression is detected in the inner myocardial layer (Fig. 1C, D), whereas the outer layer as well as the endocardium remain free of *BMP10* transcripts. In addition, *BMP10* RNA is now found in the wall of the atrium. At HH18, *BMP10* is expressed in the

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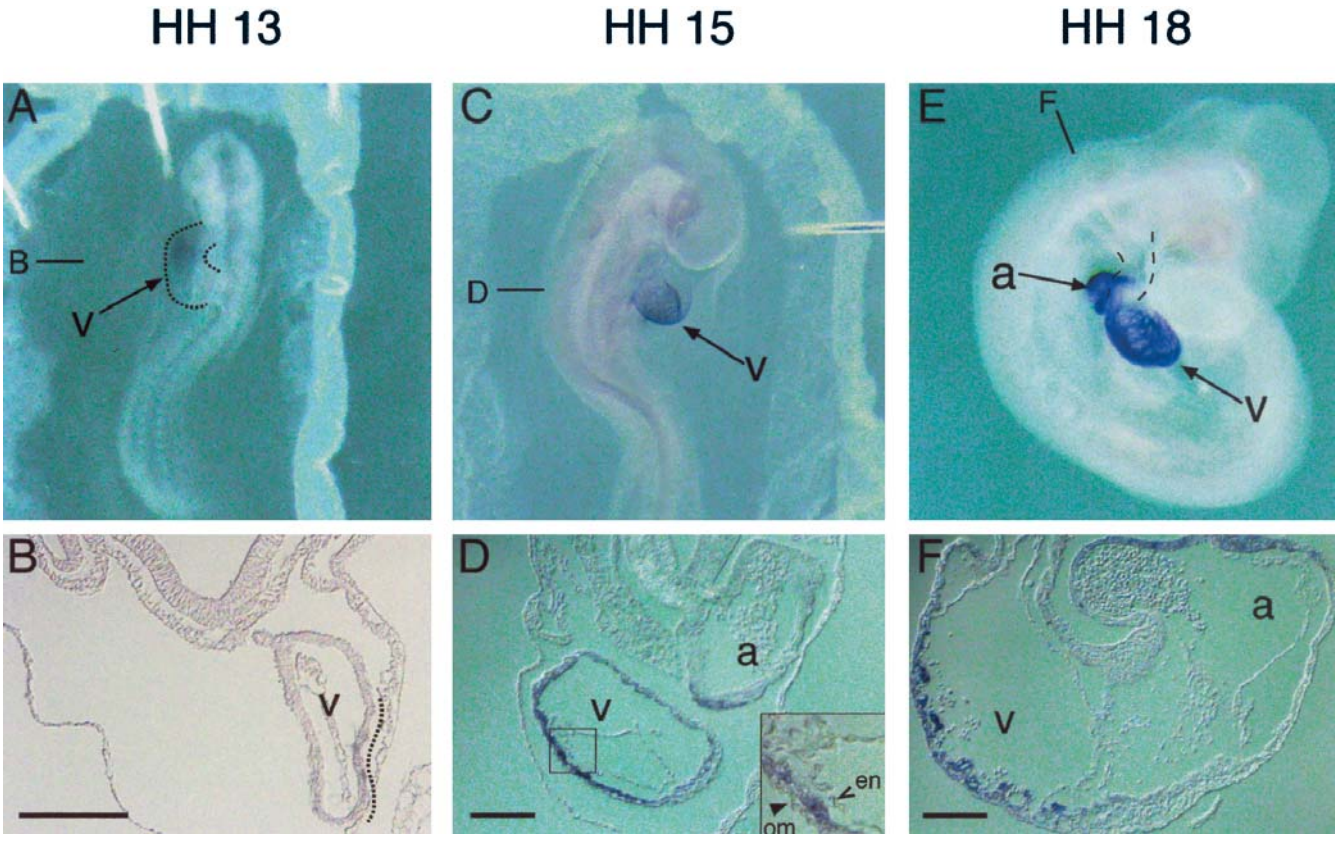


**Fig. 1A–F** Expression of *BMP10* during early stages of chick embryonic development. **A**, **C**, **E** Whole-mount in situ hybridization at HH13, HH15 and HH18 shows expression of *BMP10* exclusively in the embryonic heart (*v* ventricle, *a* atrium). In **A**, the shape of the heart is indicated by a *dotted line*. In **E**, the outflow tract is indicated by a *dashed line*. The level of the sections shown in **B**, **D**, and **F** is indicated. **B**, **D**, **F** Transversal sections demonstrate that the staining is confined to the inner myocardial layer only. The outer myocardial layer (*om*, *black arrowhead*) and the endocard (*en*, *open arrowhead*) are free of *BMP10* transcripts. Chick embryos were staged according to Hamburger and Hamilton (1951). The cloned *BMP10* cDNA fragment was used to generate antisense RNA. The expression was analyzed by whole-mount in situ hybridization essentially as described by Wilkinson (1992) but adapted as follows. Tissues were permeabilized 3×15 min in a buffer containing 0.1% SDS, 1% NP40, 0.5% Desoxycholat, 150 mM NaCl, 1 mM EDTA, 50 mM Tris pH 8.0 before the prehybridization. The pre-incubation steps before the hybridization and the antibody binding were extended to 8 h. The hybridization and the first two washing steps were performed at 70°C. The embryos were washed for 3 days in maleic acid buffers and 2 days in NTMT to remove all traces of antibody. Paraffin sections were obtained from embryos stained after whole-mount in situ hybridization. *Bar* =100 $\mu$m

trabeculating myocardial tissue of both the ventricle and the atrium (Fig. 1E, F).

Whole-mount in situ hybridization of isolated hearts from embryos ranging from HH21 to HH32 shows that the principle of this spatial pattern persists in all investigated stages (Fig. 2A–H). Expression in the ventricles of the heart is confined to the innermost trabeculae, whereas the compacting myocardial tissue on the outer side is free of *BMP10* RNA. The expression in the ventricular trabeculae becomes very weak at stage HH32 (Fig. 2G), and is barely detectable on sections at day 7 of development. During the morphogenesis of the atriae, high *BMP10* expression becomes restricted to the auricles, i.e. the trabeculated part of the atrial myocardium (HH32; Fig. 2H). *BMP10* transcripts are not present in the interventricular and interatrial septa and in the myocardium of the atrioventricular canal, where the endocardial cushions and the atrioventricular valves will form. Here, no trabeculated structures will develop. The outflow tract, also consisting of non-trabeculated myocardial tissue, is free of *BMP10* transcripts.

In conclusion, for the chicken *BMP10* gene we describe an expression pattern which is much more restricted than that found for other genes of the large BMP family. The pattern is highly conserved between chick and mouse embryos (Neuhaus et al. 1999). The extreme restriction to the inner myocardial layer implicates a strong correlation with the process of trabeculation. Trabeculation as well as *BMP10* expression occur only in myocardium derived from the primary, paired heartfield. The areas of *BMP10* expression in the ventricle correspond to the region where the heart chambers "balloon" out from the bended heart tube according to the ballooning model discussed by Christoffels et al. (2000).

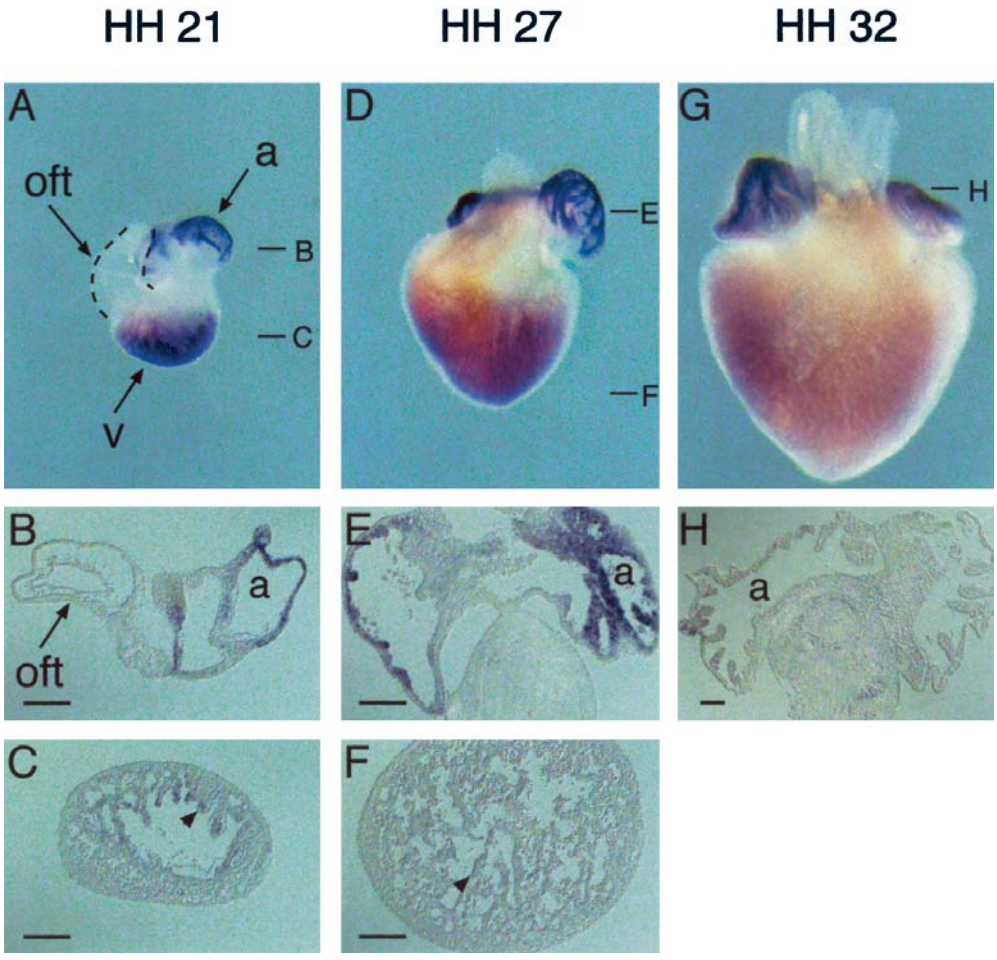


**Fig. 2A–H** Expression of *BMP10* in the hearts during late stages of chick embryonic development. **A**, **D**, **G** Whole-mount in situ hybridization of isolated hearts at HH21, HH27 and HH32 show that *BMP10* RNA is present in the ventricles (*v*) and in the atria (*a*). The outflow tract (indicated by *dashed line*, *oft*) is free of *BMP10* transcript. The level of the sections is indicated. **B**, **C**, **E**, **F**, **H** Sections demonstrate that the staining becomes confined to the atrial wall and to the innermost trabeculae (*arrowheads*) of the ventricles. *Bar* =100 $\mu$m

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