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Asymmetric expression of Syndecan-2 in early chick embryogenesis

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Abstract

Chicken *Syndecan-2* (*cSyndecan-2*) is the homologue of *Xenopus Syndecan-2*, a member of the heparan sulfate proteoglycan family with an important role in left–right patterning in frog embryos. A relationship to LR asymmetry in other species has not been reported. We show that *cSyndecan-2* is expressed throughout the primitive streak between st. 1 and 3 in the chick embryo, and is restricted to the rostral and caudal tips of the primitive streak at st. 4. It displays distinct left–right asymmetry, being expressed in the right side of Hensen's node at st. 5. The asymmetric expression of *cSyndecan-2* is maintained around the node between st. 5 and 7. At early somite stages, somites and neural folds express *cSyndecan-2*. The somite expression disappears by st. 11, but strong expression in the neural tube continues. Our data reveal a new asymmetric transcript in the chick embryo and indicate that in contrast to protein-level asymmetries, which underlie syndecan-2 function in *Xenopus*, chick *syndecan-2* exhibits asymmetry at the mRNA level.

Keywords: Syndecan-2; Embryogenesis; Left–right asymmetry; Chick

1. Results and discussion

Syndecans are a family of transmembrane heparan sulfate proteoglycans (HSPGs) with highly conserved cytoplasmic domains and heparan sulfate glycosaminoglycans covalently attached to their extracellular domain. Genes in this family have been proposed to play essential roles during development (Rapraeger, 2001). Syndecan-2 is known to be an important component of left–right patterning in early *Xenopus* embryos and is a Vg1 cofactor that regulates transduction of Vg1-related signals (Kramer et al., 2002; Kramer and Yost, 2002), one of the most critical signaling factors in early embryogenesis (Seleiro et al., 1996). *Syndecan-2* gene expression and its function in early embryogenesis have been studied in frog, where the Syndecan-2 asymmetry is observed at the post-translational level (Kramer et al., 2002; Kramer and Yost, 2002; Teel and Yost, 1996). Because of the importance of left–right asymmetry in embryonic patterning (Cooke, 2004) and the considerable current controversy about the divergence of early asymmetry mechanisms among different species (Burdine and Schier, 2000; Levin, 2003; Yost, 2001), we characterized *Syndecan-2* expression in the chick embryo during stages at which asymmetry is being elaborated.

We cloned *cSyndecan-2* based on homology to existing *Syndecan* clones (Chen et al., 2002). *cSyndecan-2* expression was weakly expressed at the posterior margin at st. 0 (Fig. 1A). In situ hybridization analysis detected *cSyndecan-2* expression at the base of the primitive streak at st. 1, and it is expressed throughout the primitive streak until st. 3 (Fig. 1B–D). Sectioning revealed *cSyndecan-2* expression in the mesendodermal layer (Fig. 1N). At st. 4, after the primitive streak has reached its maximal length, *cSyndecan-2* is expressed symmetrically at Hensen's node and at the base of the primitive streak (Fig. 1E). Interestingly, the expression pattern of *cSyndecan-2* becomes asymmetric and is expressed on the right of the node at st. 5 and 6 (Fig. 1F,G, close-up of node in Fig. 1K,L). Sectioning confirmed asymmetric expression immediately lateral to the primitive pit (Fig. 1M). This distinct asymmetric gene expression is maintained until st. 7 (Fig. 1H), but disappears by st. 8 (Fig. 1J). At st. 7, *cSyndecan-2* becomes expressed in an area coincident with the first somite (Fig. 1H; compare to expression of

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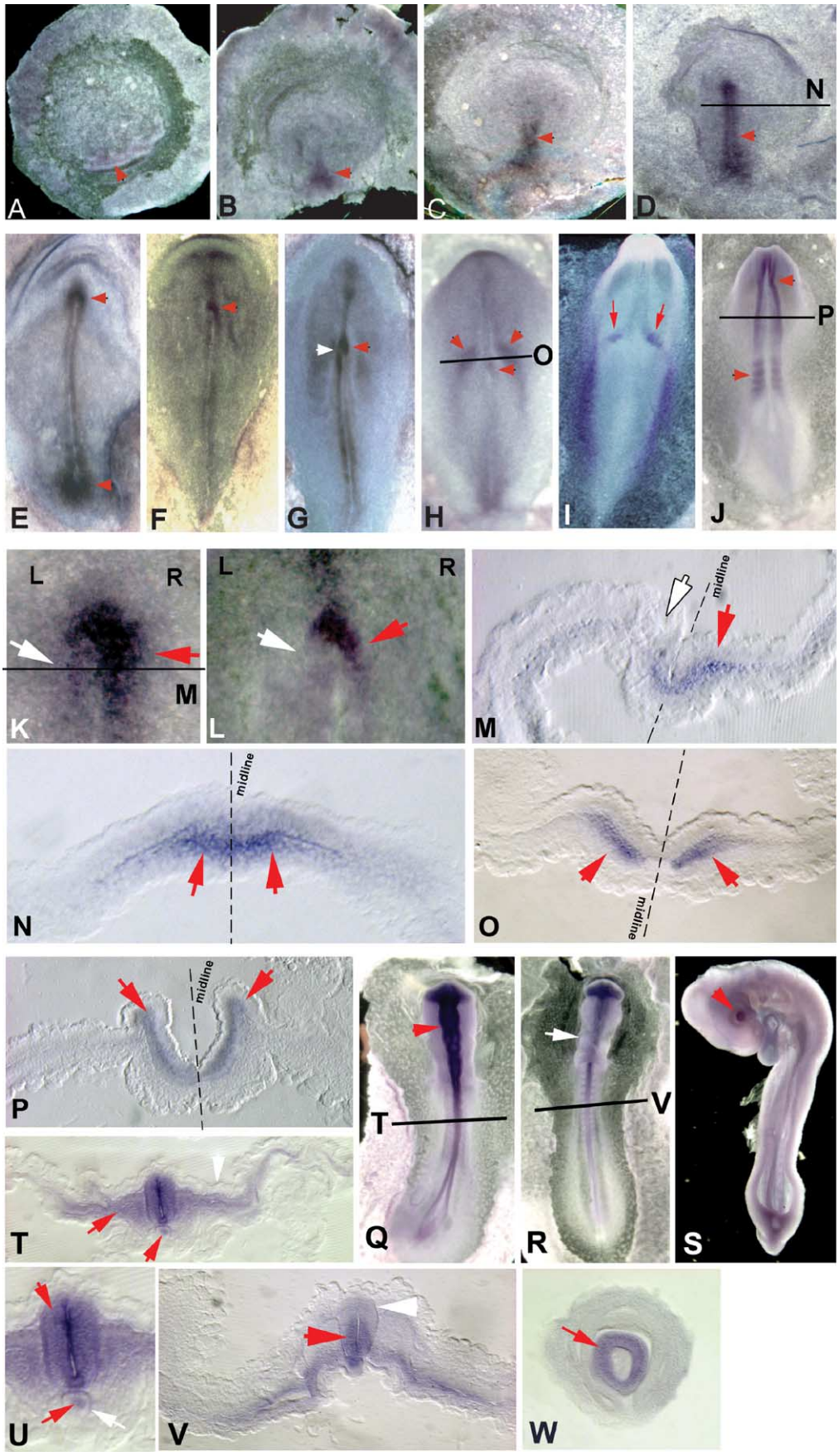
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cSerrate-2, a somite marker (Caprioli et al., 2002), Fig. 1I). Between st. 8 and 11, signal is observed in the somites and the neural folds (Fig. 1J). Sectioning confirmed expression within the mesoderm layer of the neural folds (Fig. 1O,P).

By st. 12, the neural tube expression is very strong, and the lateral plate mesoderm exhibited a lower level of expression (Fig. 1Q, section in Fig. 1T). Higher magnification reveals strong expression throughout the neural tube, and in the central, but not the peripheral, cells of the notochord (Fig. 1U). By st. 15, the neural tube staining has weakened, and no expression was observed in the heart or other tissues (Fig. 1R). Sectioning revealed that the disappearance of the *cSyndecan-2* transcript begins with the dorsal part of the neural tube (Fig. 1V, white arrow), but signal can still be observed in the ventral part of the neural tube and the notochord (Fig. 1V, red arrow). The expression of *cSyndecan-2* weakens progressively in subsequent stages; by st. 18, most of the embryo exhibited only a very low background level of expression, with the exception of the eye (Young et al., 2003), where the lens continued to express *cSyndecan-2* strongly (Fig. 1S,W). Examination of embryos until st. 23 revealed no specific stain.

Our data identify a transcription-level asymmetry in chick *Syndecan-2* at late gastrulation, and are consistent with a number of LR asymmetry pathways that occur at the protein level in *Xenopus*, but at the mRNA level in chick (Levin and Mercola, 1998, 1999).

2. Experimental procedures

The full-length sequence of *cSyndecan-2* (606 bases) was amplified from RNA extracted from st. 23 chicken embryos, using primers cSyn2F (5′-ATGCGGTGCG-TGTGGCTCGCGC-3′) and cSyn2R (5′-TTATGCA-TAAAACTCCTTAGTAG-3′) based on Gene Bank information (accession number: AF508229). This was cloned into the vector pBluescript SK(+). pBS-SK(+)–cSyndecan-2 was linearized using *Eco*RV (Takara) and transcribed with *T3* polymerase to give a DIG-labeled antisense transcript, and this was used for in situ hybridization according to standard protocols (Stern and Holland, 1993). All chick stages are according to (Hamburger and Hamilton, 1992).

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Fig. 1. Expression of chicken *Syndecan-2* at early developmental stages of the chick. (A) *Syndecan-2* is weakly expressed at the posterior margin of unincubated chick eggs. (B) At the initiation of the primitive streak, it is expressed at the base of the primitive streak. (C,D) During elongation (st. 2 and 3, respectively), *Syndecan-2* is expressed throughout the streak. Horizontal line in D indicates section shown in panel N. (E) At st. 4, *Syndecan-2* is displayed symmetrically around the node as well as at the base of the primitive streak. (F) At st. 5, *Syndecan-2* is expressed in the right side of the node. (G) At st. 6, the asymmetric expression continues at the node, and weak expression is observed in the emerging notochord. (H) At st. 7, *Syndecan-2* is expressed in the region coincident with the first somite. Horizontal line in H indicates section taken in panel O. (I) Expression of *cSerrate-2* indicates position of 1st somite for comparison with panel H. (J) At st. 8, *Syndecan-2* is expressed in the somites and the neural folds. Horizontal line in I indicates section taken for panel P. (K) Close-up of Hensen's node at st. 5^{-}. (L) Close-up of the node at st. 6. (M) Transverse section taken through the node of a st. 5 embryo. (N) Transverse section taken through the primitive streak of a st. 3 embryo. (O) Transverse section taken at the level of the first somite at st. 7. (P) Transverse section taken through the closing neural tube at st. 8^{+}. (Q) At st. 12, strong staining is observed throughout the neural tube. (R) By st. 15, staining has weakened and no signal is observed in the heart or ventral tissues. (S) At st. 18, a low level of background signal is present, but the eye expresses *cSyndecan-2* specifically. (T) Transverse section through a st. 12 embryo confirms expression throughout the neural tube and in the lateral plate mesoderm. (U) Close-up of the section in T reveals stain in the central, but not in the peripheral cells of the notochord. (V) Transverse section through a st. 15 embryo reveals signal in the ventral, but not dorsal, cells of the neural tube. (W) Saggittal section through the eye of a st. 18 embryo reveals specific *cSyndecan-2* expression in the lens. Red arrows indicate expression; white arrows indicate lack of expression. Dashed lines indicate embryonic midline. Sections are shown dorsal side upwards. All wholemount embryos are shown dorsal-side upwards, except for the embryo in R, which is ventral side upwards.

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