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A CHICKEN *WNT* GENE, *WNT-11*, IS INVOLVED IN DERMAL DEVELOPMENT+

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Summary: We have isolated a new member of the *Wnt* gene family, *Wnt-11*, from chick embryo cDNA library and examined the expression pattern during embryogenesis by *in situ* hybridization. The *Wnt-11* gene encodes Cys-rich secretory protein distantly related to *Wnt-1* through *Wnt-8* from the mouse and *Xenopus*. Expression of the *Wnt-11* gene became evident at stage 14 in the dorsolateral region of somites and gradually restricted to the dermatome at stage 19 and later. In contrast to the other *Wnt* genes, *Wnt-11* was not expressed in the neuroepithelium throughout stages 14-26. At stage 24 and later, *Wnt-11* was expressed in the subectodermal mesenchyme of the limb and feather buds. The unique expression pattern of *Wnt-11* in the paraxial mesoderm and dermatome suggests that *Wnt-11* may play an important role in dermal development.
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Wnt-1 was initially identified as a gene activated by proviral insertion of the mouse mammary tumor virus, and later recognized as a vertebrate counterpart of a *Drosophila* segment polarity gene *wingless* (1,2). The *Wnt* gene encodes Cys-rich secretory protein and is expressed in a variety of adult and embryonic tissues, implicating an important role as a signaling factor in the cell-cell interaction during embryogenesis. Targeted disruption of the *Wnt-1* gene leads to severe deformities in the developing midbrain (3-5), and ectopic expression of the *Wnt-1* and *Wnt-8* genes leads to formation of the complete secondary axis in *Xenopus* embryos (6-8).

To study the role of *Wnt* genes during chick embryogenesis, we isolated several members of the *Wnt* gene family by polymerase chain reaction (PCR) using degenerate primers. We identified a novel member from chick embryos, designated *Wnt-11*, which

+Accession number for the chicken *Wnt-11* gene is D31901 in GSDB/DDBJ/EMBL/NCBI databases.

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is distantly related to other members of the *Wnt* family. The expression pattern was examined by whole-mount *in situ* hybridization of chick embryos, and intense expression was observed in the dorsolateral region of the somite, which later becomes connective tissue of the dorsal skin. The unique expression pattern of the *Wnt-11* gene in the dermatome suggests a distinct role of *Wnt-11* as an inductive signal for dermal development.

MATERIALS AND METHODS

Reverse transcription of the total RNA from 4-day chick embryos was carried out using MMLV reverse transcriptase (BRL). PCR amplification of the cDNA using Wnt specific primers was described previously (9). The PCR products were cloned into pBluescript SK(+), and identified by nucleotide sequencing.

One of the PCR clones encoding chicken *Wnt-11* was used as a probe for hybridization screening of chick embryo cDNA library to obtain full-length cDNA. Filter hybridization was carried out as described previously (10). cDNAs from the recombinant phages were subcloned into pGEM7Zf(+) and M13mp18/mp19 after restriction digestion, and sequenced in both orientations by dideoxy chain-termination method.

Whole-mount *in situ* hybridization was performed as described previously (11). Digoxigenin-labeled cRNA probe was prepared according to manufacturer's protocol (Boehringer). Sense strand probe was used for negative control, and no hybridization signal was detectable with the sense probe. After hybridization, the embryo was incubated with the antibody coupled to alkaline phosphatase, and then with BCIP/NBT for detection. Hybridized embryos were sectioned and examined for the presence of expression signals as described (11). Photographs were taken by using an Olympus model BH-2 microscope. Chick embryos were staged according to Hamburger and Hamilton (12).

RESULTS

Cloning of *Wnt-11* cDNA: By screening cDNA library using the chicken *Wnt-11* probe, we obtained 1.83 kb cDNA encoding full-length Wnt-11 protein. Sequence comparison of the deduced protein reveals that the chicken *Wnt-11* gene is distantly related to *Wnt-1* through *Wnt-8*, and closely related to *Wnt-11* identified in the mouse and *Xenopus* (Fig. 1). Chicken Wnt-11 protein exhibits 84% identity to mouse Wnt-11 and 67% identity to *Xenopus* Wnt-11 (13), thus identifying as a chicken cognate of the *Wnt-11* gene.

Developmental expression of the *Wnt-11* gene: We performed whole-mount *in situ* hybridization to find out the expression pattern during chick embryogenesis. *Wnt-11* signals were first detectable in the lateral plate mesoderm at stage 14, and became evident at stage 17 and later (Fig. 2A). The mesodermal expression was delimited to the dorsal region of somites. This region later becomes dermatome, a primordium of mesenchymal connective tissue of the dorsal skin. Expression in the somite was most evident at stages 17 to 21 (Figs. 2B, 2C), and

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                                                                *
Wnt-11 (Gg)  M---KPSPQFF-LAAFLSL-ILQTGICYGI--KWIALSKTPSSLALNQTQHC------KQLEGLVVSQVQ   57
Wnt-11 (Mm)  .---RAR..VC-E.LLFA.-A.H..V....---..L......AA.....------.....SA...   57
Wnt-11 (Xl)  .---A.TRHWV-TPLL.-.-C-CS...GA.--Q.LG.TVNG.RV.W.ESE..------RL.D...PD.S.   55
 Wnt-1 (Mm)  .GLWALL.SWVSTTLL.A.TA.PAALAANSSGR.WGIVNIA..TN.LTDSKSLQLVLEPS.QL.SRK.RR   70

                                    o          *o
Wnt-11 (Gg)  LCRSNLELMQTIIQAAREVIKTCRKTFSDMRWNCSSIELAPNYLLDLERGTRESAFVYALSAAAISHTIA  127
Wnt-11 (Mm)  .........R..VH...GAM.A..RA.A.................................T......  127
Wnt-11 (Xl)  ..KR......SVVN..KQTKL..QM.L.........V.N..SFTP..SK...........AS.TL.....  125
 Wnt-1 (Mm)  .I.Q.PGILHSVSGGLQSAVRE.KWQ.RNR....PTAPGPHLFGKIVN..C..T..IF.ITS.GVT.SV.  140

               o       o o                  o
Wnt-11 (Gg)  RACTTGDLPGCSCGPIPGETPGPGYRWGGCADNLNYGLIMGSKFSDAPMKMKKS-GSQANKLMHLHNSEV  196
Wnt-11 (Mm)  ....S.........V...P....N.........S...L..A........V..T-........R......  196
Wnt-11 (Xl)  ...AS.E..T....AT.A.V..T.F.....G...H...N...A.V.....SS..A.T..T.I.N...NA.  195
 Wnt-1 (Mm)  .S.SE.SIES.T.DYRRRGPG..DWH....S..IDF.RLF.RE.V.SGE.------.RDLRF..N...N.A  205

                         o o       o    o
Wnt-11 (Gg)  GRQVLKASLEMKCKCHGVSGSCSIKTCWKGLQELRDIALDLKNKYLSATKVV--HR--PMGTRKYLV---  259
Wnt-11 (Mm)  ...A.R....T.............R.........Q.V.A...TR.........--..--......H..---  259
Wnt-11 (Xl)  .....MD...T.............V.........D.PH..NE..S...G....I--..--QT...RQH.---  258
 Wnt-1 (Mm)  ..TTVFSEMRQE.....M....TVR...MR.PT..AVGDV.RDRFDG.SR.LYGN.GSNRAS.AE.LRLE  275

                                       o                  o    *    o   oo             o
Wnt-11 (Gg)  PKDIDIRPVKETELIYLQSSPDFCMKNEKVGSHGTQDRQCNKTSNGSDSCDLMCCGRGYNPYMDKVVERC  329
Wnt-11 (Mm)  ...L......DS..V.....................................................T.R.....  329
Wnt-11 (Xl)  .REL.....R.S..V..V....Y.T..P.L..Y.....L.....V.....N.........A.TETI....  328
 Wnt-1 (Mm)  .E.PAHK.PSPHD.V.FEK..N..TYSGRL.TA..AG.A..SS.PAL.G.E.L.....HRTRTQR.T...  345

               o    oo  o  o        o
Wnt-11 (Gg)  HCKYHWCCYVTCKKCERTVERYVCK  354
Wnt-11 (Mm)  ............RR...........  354
Wnt-11 (Xl)  Q.........M..............  353
 Wnt-1 (Mm)  N.TF....H.S.RN.TH.RVLHE.L  370
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Fig. 1. Alignment of chicken Wnt-11 with other Wnt-11 members. Gg, chicken; Mm, mouse; Xl, *Xenopus leavis*. Amino acid sequences of mouse Wnt-1 (17), mouse Wnt-11 (18) and *Xenopus* Wnt-11 (13) are aligned, showing conserved residues with chicken Wnt-11 by dots. Gaps are introduced to improve alignment and indicated by dashes. Potential signal peptide sequence is double-underlined. Potential sites for N-glycosylation are indicated by asterisks, and well-conserved Cys residues are shown by open circles above the chicken Wnt-11 sequence.

correlated to the formation of dermatome. Intermittent expression in the somite along the body plan was clearly visible at these stages, and slightly intensified in the anterior region. At stage 24 and later, *Wnt-11* expression was dispersed laterally within the dermatome (Fig. 2D). The hybridized embryo was sectioned to confirm precisely the region of *Wnt-11* expression. Transverse sections of the embryo at stage 20 showed that *Wnt-11* was expressed in the dorsal region of the dermatome (Fig. 2E). Mesenchymal expression was observed beneath the epithelium on parasagittal sections at stage 24 (Fig. 2F). *Wnt-11* expression was dispersed in the dermal mesenchyme surrounding sclerotome, a primordium of vertebral cartilage. No expression signal was detected in the spinal cord and diencephalon throughout stages 14 to 24, in contrast to the other *Wnt* genes, such as chicken *Wnt-4* and *Wnt-2* (11).

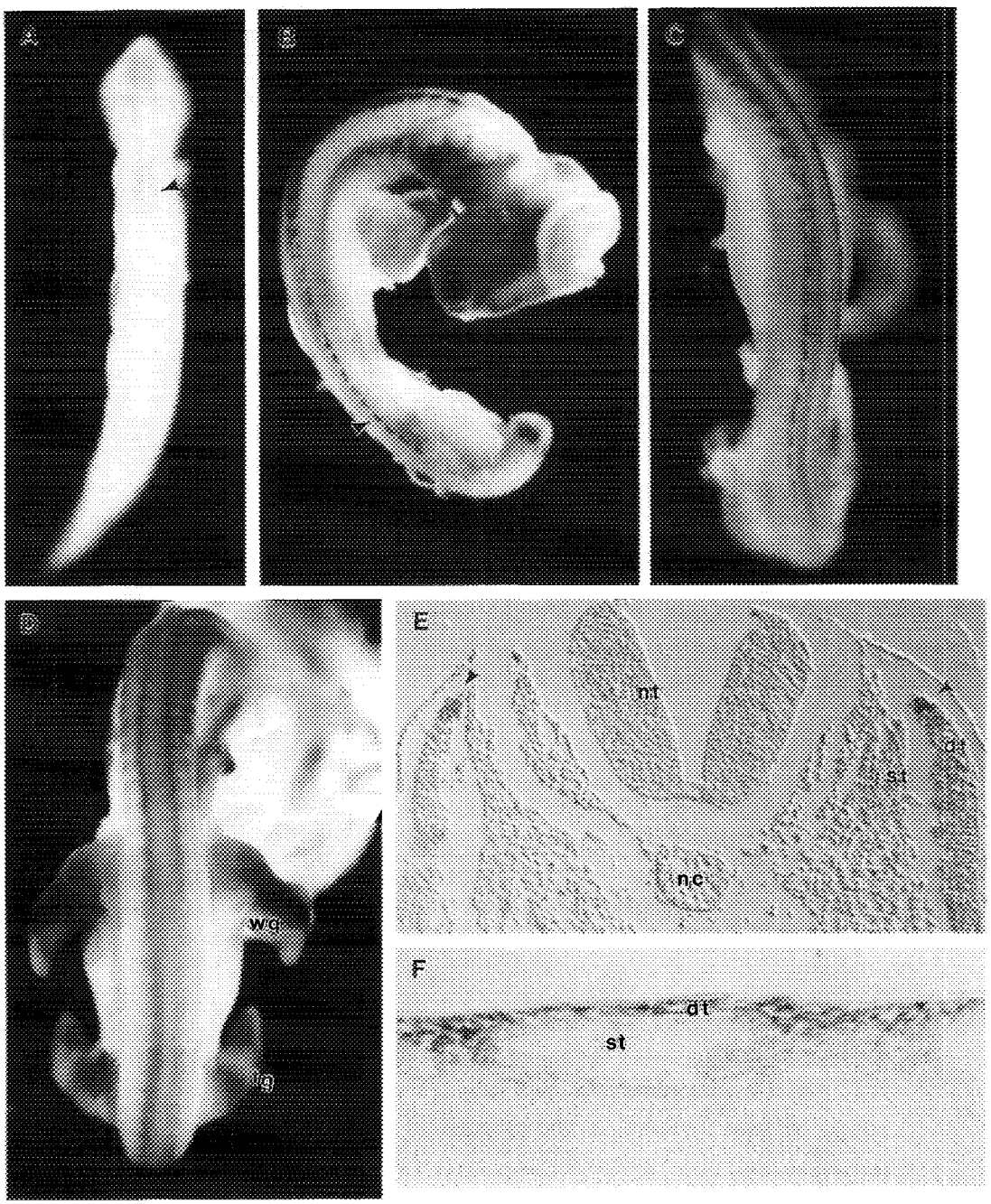
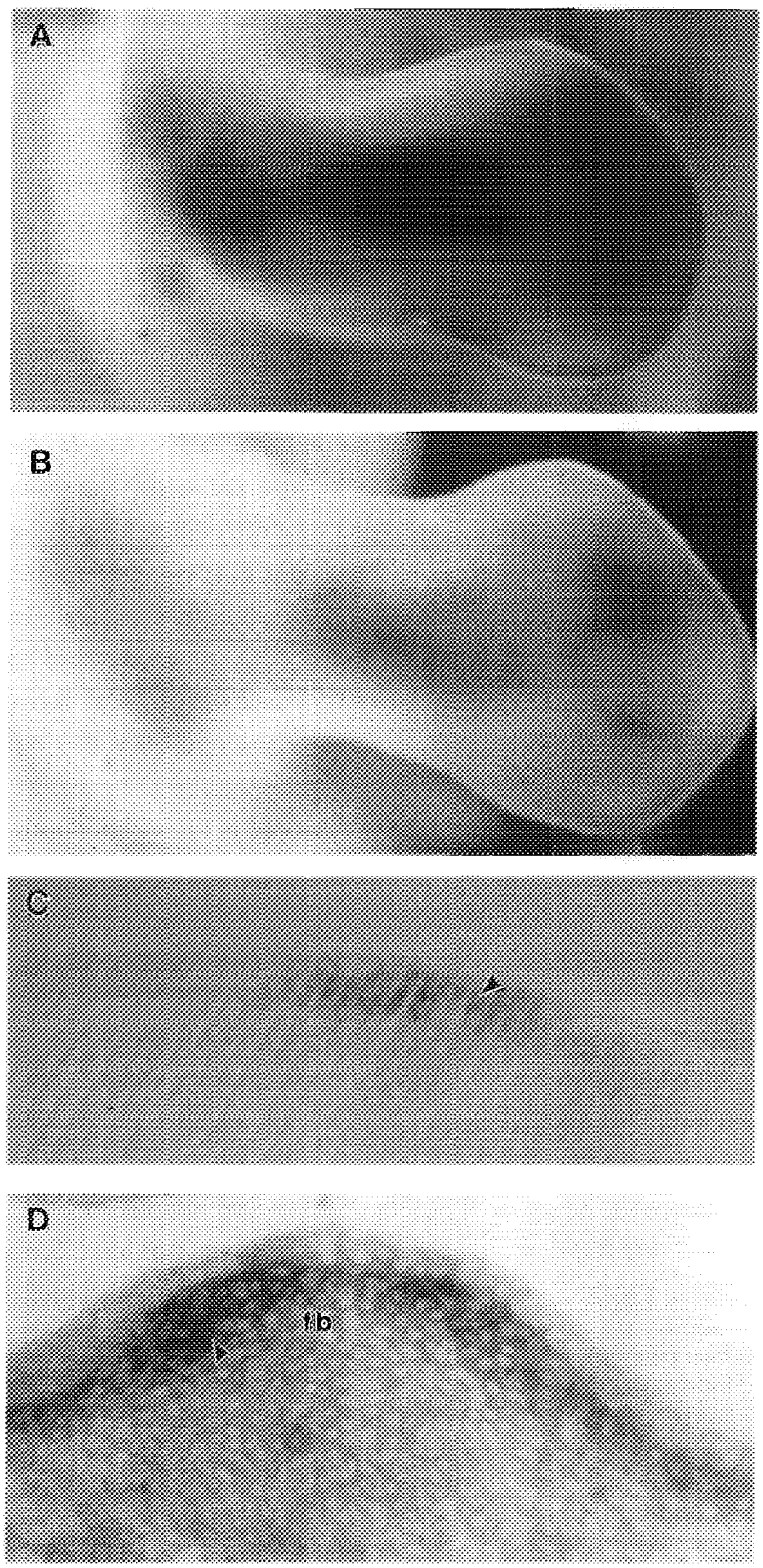


Fig. 2. *Wnt-11* expression in the chick embryo detected by whole-mount *in situ* hybridization. (**A**) Dorsal view of the stage 14 embryo. *Wnt-11* transcripts are detectable in the paraxial mesoderm along the body plan (arrowhead). (**B**) Lateral view of the stage 19 embryo. Strong signals are detectable in the dermatome (arrowhead), primordium of axial dermis. Staining in the head and tail bud region is an artifact. (**C**) Dorsal view of the stage 21 embryo. Intermittent *Wnt-11* signals in the dermatome are intensifying anteriorly. (**D**) Dorsal view of the stage 26 embryo. Expression in the dermatome is dispersed laterally, and wing (wg) and leg (lg) buds showed intense expression in the dorsal region. (**E**) Transverse section of the whole-mount hybridized embryo at stage 20. The *Wnt-11* expression is detected in the dorsal region of the



(3)

At stages 24 to 28, intense signals were clearly detectable in the wing and leg buds (Figs. 2D, 3A, 3B). Although expression in the limb bud did not overlap dermatomal expression, *Wnt-11* signals were dorsally restricted within the limb bud. Signals were only detectable in the limb mesenchyme, but not in the limb ectoderm, as observed in the transverse sections of the hybridized embryo (Fig. 3C). *Wnt-11* was also expressed in the feather bud. At stages 35-36, signals were detectable in the mesenchyme adjacent to the feather ectoderm (Fig. 3D). The *Wnt-11* gene may play a role in the formation of dermal structure both limb and feather buds.

DISCUSSION

We have identified a new member of the *Wnt* gene family, *Wnt-11*, from chick embryo cDNA library and characterized the temporal and spatial expression pattern during embryogenesis. The *Wnt-11* gene shows the entirely different expression pattern from other *Wnt* members. For example, *Wnt-1*, *Wnt-2*, *Wnt-3*, *Wnt-4*, *Wnt-5*a, and *Wnt-7b* were expressed in the dorsal or ventral neuroepithelium of the diencephalon and spinal cord in the mouse and chicken embryos (11,14-16). As demonstrated here, *Wnt-11* expression was not observed in these ectoderm-derived cells throughout early embryogenesis. *Wnt-11* was not expressed in the spinal cord either, thus constituting distinct characteristics for a *Wnt* gene.

In the *Xenopus* embryos, *Xwnt-11* was expressed in the somites at early tail bud and tadpole stages (13). Chicken *Wnt-11* was also expressed in the dermatome structure of the somite, which is derived from paraxial mesoderm and associated with axial dermis formation. None of the other *Wnt* family members is expressed in the paraxial mesoderm, suggesting a unique role of *Wnt-11* in dermal development. However, no significant expression in the sclerotome and myotome implicates involvement of *Wnt-11* in determination of developmental cell fate stemmed from the somite. The temporal expression pattern of *Wnt-11* in the paraxial mesoderm correlates with the formation of dermatome structure.

The expression pattern of *Wnt-11* in the limb bud also differs from *Wnt-5a*, *Wnt-7a*, *Wnt-3*, and *Wnt-6*, which are expressed in the mouse limb bud (15,16). These

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dermatome (arrowhead). dt, dermatome; nc, notochord; nt, neural tube; st, sclerotome. (**F**) Parasaggital section of the whole-mount hybridized embryo at stage 24. *Wnt-11* expression is dispersed in the dorsal mesenchyme beneath the epithelium.

Fig. 3. *Wnt-11* expression in the limb and feather buds. (**A**) Wing bud at stage 26. (**B**) Leg bud at stage 26. (**C**) Transverse section of the whole-mount hybridized wing bud at stage 28. *Wnt-11* expression is observed in the dorsal mesenchyme adjacent to the limb ectoderm (arrowhead). (**D**) Feather bud (fb) at stage 35. *Wnt-11* signals are detectable in the feather mesenchyme (arrowhead).

Wnt genes are expressed in the dorsal and/or ventral ectoderm of the limb bud. In contrast, *Wnt-11* was not expressed in the limb ectoderm, but expressed in the limb mesenchyme. Thus, the expression domain of *Wnt-11* is mutually exclusive to the other *Wnt* genes. *Wnt-11* may be involved in cell fate determination of the limb mesenchyme.

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