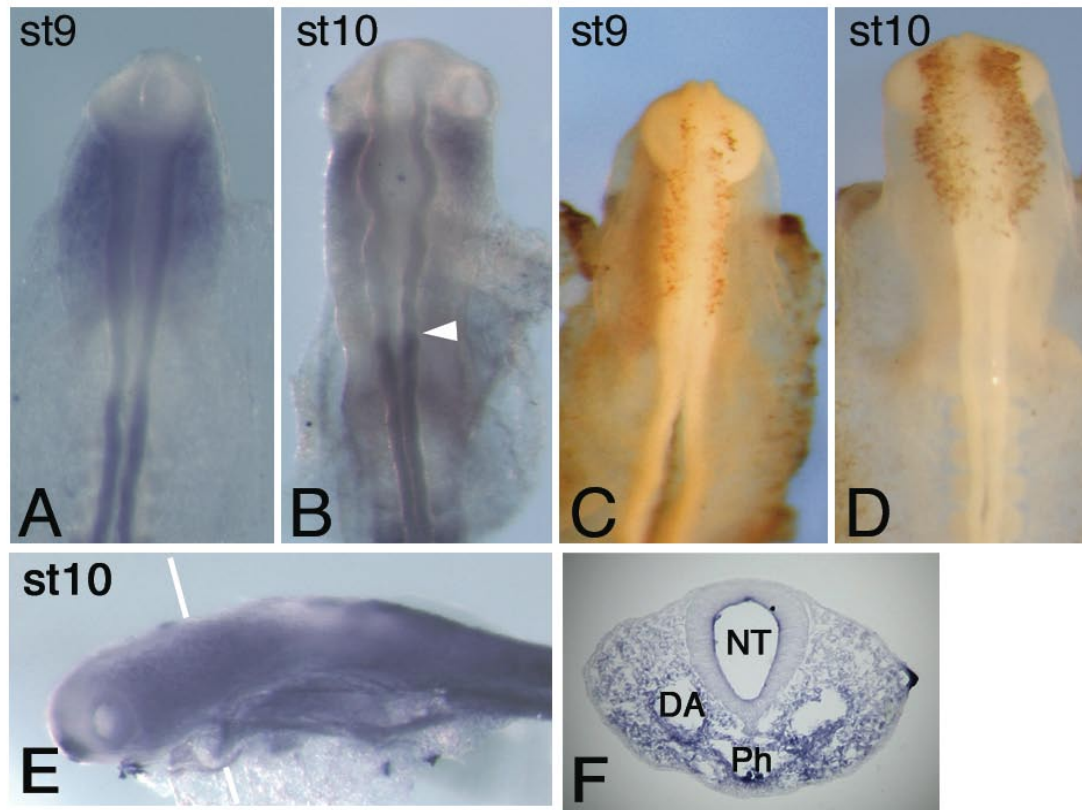


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Int. J. Biol. Sci. 2011; 7(1):87-101 - Supplementary Figure 1
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Supplementary Figure 1:

Expression pattern of chicken *HOXA3* in early stage embryos. *In situ* hybridization for *HOXA3* mRNA in st-9 (A) and st-10 (B, E, F) revealed signals not only in the region posterior to the r4/5 boundary (white arrowhead in "B") but also in the anterior neural tube and the head mesenchyme. (F) A transverse section through the white line in "E". Weak signals are seen in the neural tube and the mesenchymal cells. For comparison, neural crest cells were stained with HNK-1 antibody (C, D). NT, neural tube; DA, dorsal aorta; Ph, pharynx.



Supplementary materials and methods

RT-PCR

Fertilized chicken eggs (Yamagishi, Japan) were incubated at 38°C until st-10 or -20 [22]. St-10 embryos were cut into anterior (A) and posterior (P) tissue regions at the r2/3 boundary; and st-20 embryos, at the mid-brain/hindbrain boundary. For mouse embryos, noon of the day vaginal plugs were found was designated as embryonic day 0.5 (E0.5). Embryos at the 14-18-somite (E9.0) and 40-somite (E10.5) stages were collected and cut at r4 into anterior (A) and posterior (P) tissue regions. Tissue A contained branchial arch1, r3, and the more anterior region; and tissue P, branchial arch2, r5, and the more posterior region. Embryos at E12.5 were dissected and divided at the midbrain/hindbrain boundary into A and P tissues. Forebrain, midbrain, and surrounding tissues were in "A," and the remaining part was in "P." Total RNA was purified from each of them. After treatment with RNase-free DNaseI (Stratagene), the RNA samples of A and P tissues were subjected to RT-PCR with ReverTra Ace and KODDASH (TOYOBO). For analysis of *HOXA3* expression and *GAPDH* amplification (as a control for amount loaded), the following respective primer sets were used, each at 0.5 mM: 5'CAGCCACCTCCAGCCCCGCA3' (forward) and 5'CGGGCTCGCTTAGAGGATGCTTG3' (reverse); and

5'ACAGCCATTCTTCCACCTTTGAT3'(forward) and 5' CTATCAGCCTCTCCCACCTCCC3' (reverse). For amplification of mouse *Hoxa3* and *HPRT* as an RNA level control, the previously described primers [23, 63] were used at 0.1 and 0.5 .M, respectively. PCR reactions for *HOXA3/Hoxa3* were done for 35 cycles of 98°C for 10 sec, 65°C (for chicken samples) or 64°C (for mouse *Hoxa3*) for 2 sec, and 74°C for 20 sec); and for *HPRT*, 35 cycles of 94°C for 30sec, 64°C for 30 sec, and 74°C for 1 min. Sequencing of DNA fragments amplified with *Hoxa3/HOXA3* primers from "A" tissues revealed that these DNA had indeed been derived from *Hoxa3/HOXA3* transcripts.

Immunohistochemistry

Staining for the HNK-1 epitope, an NCC marker, was done as described by Kuratani and Kirby [64] with slight modification; i.e., for bleaching embryos, 1% H_2O_2 in PBS containing 0.3% TritonX-100 was used.