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Gene expression pattern

Expression patterns of group-I *aristaless*-related genes during craniofacial and limb development

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Abstract

Aristaless-related proteins are structurally defined by the presence of a paired-type homeodomain and an additional conserved domain, known as aristaless domain or OAR-domain. These proteins can be further categorized in three groups (Int. J. Dev. Biol., 43 (1999) 651). Group-I *aristaless*-related genes are linked to functions in the development of the craniofacial and appendicular skeleton and are expressed predominantly in the mesenchyme in stages from gastrulation through at least mid-gestation (Mech. Dev., 48 (1994) 245; Mech. Dev., 52 (1995) 51; Development, 124 (1997) 3999; Dev. Biol., 199 (1998) 11; Development, 126 (1999) 495). In view of the highly redundant character of the functions of these genes in patterning craniofacial and limb structures, we found it important to directly compare their expression patterns at critical stages of craniofacial and limb development.

Keywords: *Aristaless*-related genes; Limb development; Craniofacial development; Mouse

1. Results

1.1. Craniofacial regions

At E9.5, *Alx3*, *Alx4* and *Cart1* are highly expressed in the frontonasal process mesenchyme underlying the nasal placodes, *Cart1* expression extending more ventrally (see arrows Fig. 1A,E,I and Fig. 2A–C). At E10.5 and E11.0 they are expressed in the lateral and medial nasal process mesenchyme (see Figs. 1B–D,F–H,J–L and 2K–M), while *Cart1* expression is excluded from a dorsal region in the nasal processes (circle in Fig. 1L).

From E9.5 to E11.0, *Alx3*, *Alx4* and *Cart1* are expressed in the distal mandibular arch (see Figs. 1B,C,F,G,J,K and 2K–M,, *Alx4* expression extending more proximally and *Cart1* being expressed in a more oral domain (see Fig. 1G,K). Only *Alx4* and *Cart1* are expressed in the distal tips of the maxillary process (arrows in Fig. 1F,J).

Prx1 and *Prx2* are also expressed in craniofacial primordia but, compared to *Alx3*, *Alx4* and *Cart1*, at a lower level in the frontonasal processes (see arrows in Fig. 1N,R) and stronger in the mandibular and hyoid arch mesenchyme.

Prx1 is expressed in the frontonasal process in a continuous domain, while *Prx2* expression is interrupted in the extreme dorsal mesenchyme (circles in Fig. 1S,T). *Prx1* and *-2* are strongly expressed in the mesenchyme of the mandibular and hyoid arches, except in the most proximal regions in E9.5–E11.0 mouse embryos (see Fig. 1M–T). In addition, the expressions of *Prx1* and *Prx2* were detected in the otocyst (arrowheads in Fig. 1N,R).

At E9.5, the expression of *Prx3* was very low in the mesenchyme of the frontonasal processes and undetectable in the branchial arches (see Fig. 1U). At E10.5 and E11.0, the *Prx3* was detected in the medial nasal processes, proximally in the maxillary process and mandibular arch, and also in the cranial ganglia and the midbrain (see Fig. 1V–X).

1.2. Limbs

In E9.5 mouse embryos, the forelimbs have begun to grow out, but the hindlimb buds are only just recognizable. At E9.5, *Alx3*, *Alx4* and *Cart1* are expressed in the mesenchyme of the presumptive hindlimb buds and in the forelimb buds (see Fig. 3A–C,G–I,Y). In the presumptive hindlimb buds, *Alx3* and *Alx4* expressions appear to be restricted to more anterior regions, whereas *Cart1* expression is visible throughout (see Fig. 3A–C,Y). *Alx3* and *Alx4* are expressed anteriorly in E9.5 forelimb bud mesenchyme (see Figs. 2F,G and 3G,H,Y). *Alx3* is also expressed proximally in the posterior forelimb mesenchyme and in the flank. Interestingly, in contrast to the published results (Zhao et al., 1994, 1996), we do detect *Cart1* expression in the early limb bud. Expression was found proximally in both anterior and

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posterior E9.5 limb bud mesenchyme (arrows in Fig. 3I). The *Cart1* expression in the anterior limb bud mesenchyme may explain the enhanced polydactyly in *Alx4*/*Cart1* double mutants (Qu et al., 1999). In E10.5 limb buds, *Alx3* and *Alx4*

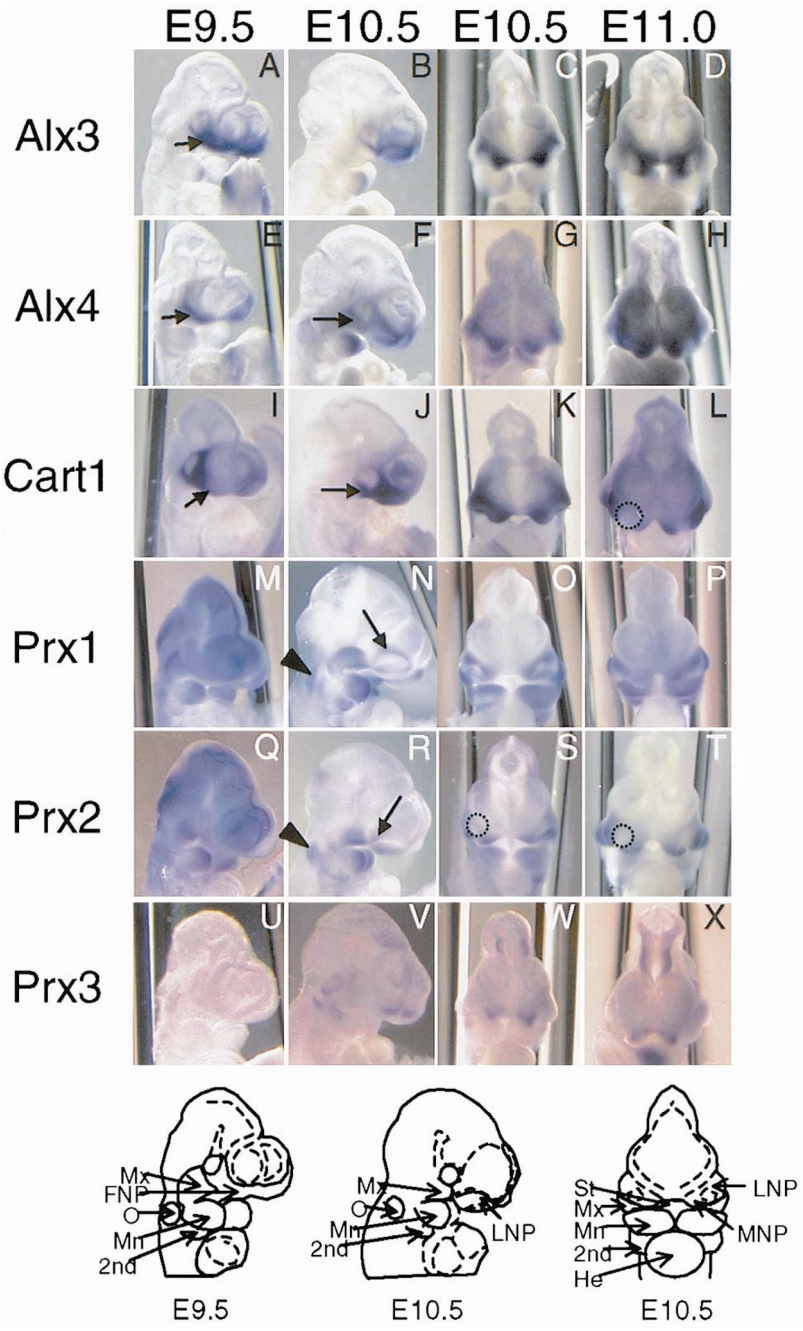

Fig. 1. Expression of *Alx3*, *Alx4*, *Cart1* and *Prx1–3* in whole mount E9.5–E11.0 mouse embryos. The first column shows semilateral views on E9.5 embryos, the second column shows the lateral views on E10.5 embryos, the third column shows the frontal views on E10.5 embryos and the fourth column shows the frontal views on E11.0 embryos. Subsequent rows show expression patterns of *Alx3*, *Alx4*, *Cart1*, *Prx1*, *Prx2* and *Prx3*, respectively. Diagrams illustrate crucial structures during craniofacial development in E9.5 and E10.5 mouse embryos. Caudal ends of E10.5 and E11.0 embryos were removed to allow free view on craniofacial regions. Arrows in (A,E,I) point towards the expressions of *Alx3*, *Alx4* and *Cart1*, respectively, in the mesenchyme underlying nasal placodes. Arrows in (F,J) show expressions of *Alx4* and *Cart1*, respectively, in the maxillary process. Arrows in (N,R) point towards the expressions of *Prx1* and *Prx2*, respectively, in the nasal processes. Arrowheads in (N,R) point towards expressions of *Prx1* and *Prx2*, respectively, in otocyst. Circles in (L,S,T) show the region of nasal process mesenchyme from which expressions of *Cart1* and *Prx2*, respectively, are excluded. Staining in dorsal regions of the hindbrain as observed in (D) should be considered as an artefact. Note expression of *Prx3* in mesencephalon in panel (V) and trigeminal and facio-acoustic complex in panel (X). Abbreviations: FNP, frontonasal process; He, heart; LNP, lateral nasal process; Mn, mandibular arch; MNP, medial nasal process; Mx, maxillary process; O, otocyst; St, stomodaeum; 2nd, 2nd branchial arch.

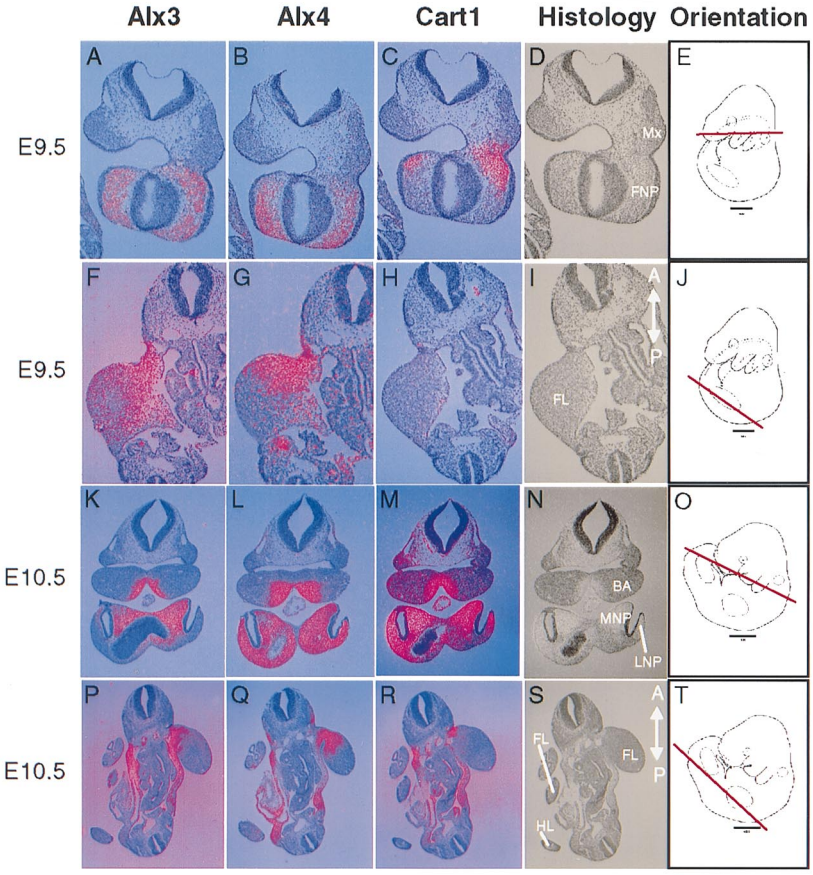

Fig. 2. Expressions of *Alx3*, *Alx4* and *Cart1* in nearby serial transversal sections of E9.5 and E10.5 mouse embryos. The first three columns show expressions of *Alx3*, *Alx4* and *Cart1*, respectively. The fourth column shows sections with locations of important structures. In the fifth column the red line shows the approximate plane of sectioning. The subsequent rows show nearby sections through E9.5 frontonasal process (A–E), E9.5 forelimb bud (F–J), E10.5 facial processes (K–O) and E10.5 forelimb bud (P–T). Arrows in (I,S) show approximate antero-posterior polarity of section. Abbreviations: BA, mandibular arch; FL, forelimb; FNP, frontonasal process; HL, hindlimb; LNP, lateral nasal process; Mx, maxillary process.

are expressed anteriorly and *Cart1*, barely detectably, both posteriorly and anteriorly (Figs. 2P and 3S–U,Y).

Prx1 and *Prx2* are also expressed at E9.5 both in fore- and presumptive hindlimb bud mesenchyme in a broad region along the limb margin (see Fig. 3D,E,J,K,Y). In the forelimb buds, expression is graded at this stage and at E10.5, with highest levels nearer to the epithelium (see Fig. 3P,Q,V,W,Y).

The expression patterns of *Prx1* and *Prx2* overlap with, but are considerably different compared to those of *Alx3*, *Alx4* and *Cart1*. The shortening of the zeugopod in *Prx* mutants (Ten Berge et al., 1998b; Lu et al., 1999) should be attributed to a defect in the cartilage growth and differentiation, when *Prx1* and *Prx2* are expressed at E12.5 in the mesenchyme surrounding condensations and at E14.5 in the perichondrium (see Fig. 4).

The *Prx3* expression is at E9.5, detected only in the forelimb bud (see Fig. 3F,L,Y) in a pattern nearly complementary to that of *Prx1* and *Prx2* at that stage, since it is excluded from the most distal limb margin and highly expressed in more proximal areas. At E10.5, *Prx3* is expressed both in fore- and hindlimbs in a similar pattern as in the E9.5 forelimb buds, but now expression seems to be down-regulated in a proximo-anterior region where *Alx3*, *Alx4* and *Cart1* are expressed (see Fig. 3R,X,Y).

2. Conclusions

The expression patterns described suggest a classification of the *aristaless*-related group-I genes in three subgroups, which is consistent with the similarities in the gene structure and results from functional studies as far as available. One group consists of *Alx3*, *Alx4* and *Cart1*, another of *Prx1* and *Prx2* and the last of *Prx3*.

3. Experimental procedures

3.1. Embryos and in situ hybridization

All procedures were carried out as described in Leussink et al. (1995) and Ten Berge et al (1998a,b). All animal experiments were conducted under the approval of the

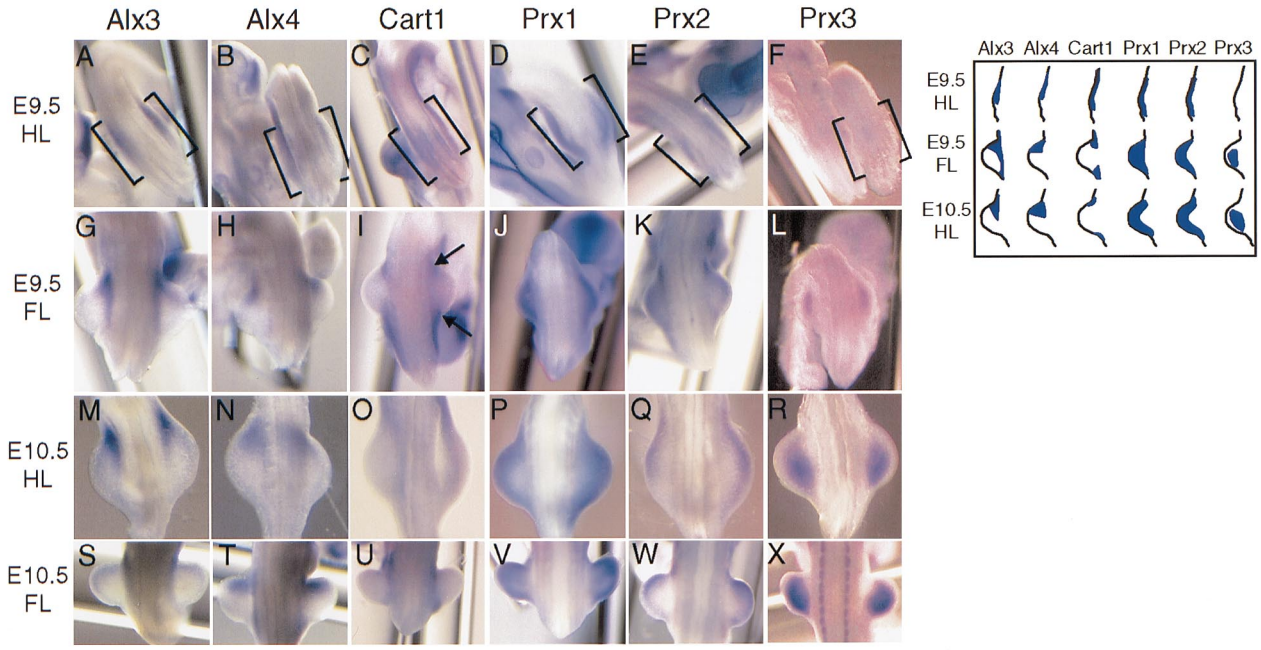

Fig. 3. (A–X) Expression of *Alx3*, *Alx4*, *Cart1* and *Prx1–3* in E9.5 and E10.5 whole mount mouse fore- and hindlimbs. Subsequent columns show expression patterns of *Alx3*, *Alx4*, *Cart1* and *Prx1–3*, respectively. The first row shows E9.5 presumptive hindlimb buds, the second row shows expression in E9.5 forelimb buds, the third row shows expression in E10.5 hindlimb buds and the fourth row shows expression in E10.5 forelimb buds. Brackets in the first row delineate the region of presumptive hindlimb bud. Note the expression of *Prx3* in dorsal root ganglia in panel (X). (Y) Diagrams showing summary of expression data in limbs.

animal care committee of the KNAW (Royal Dutch Academy of Arts and Sciences).

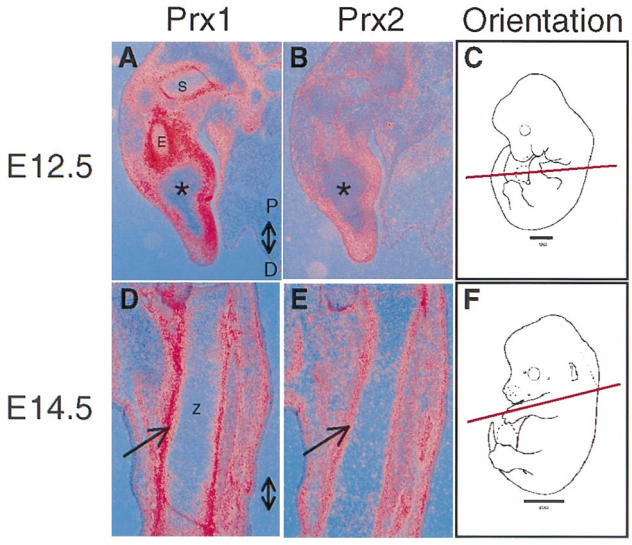

Fig. 4. Expression of *Prx1* (A,D) and *Prx2* (B,E) in nearby serial transversal sections of E12.5 (A,B) and E14.5 (D,E) mouse embryo forelimbs. Asterisks in (A,B) are positioned in mesenchymal condensations of the forelimb zeugopod. Arrows in (D,E) point towards the expressions of *Prx1* and *Prx2*, respectively, in the perichondrium of the forelimb zeugopod. The red lines in the diagrams in (C,F) show the approximate plane of sectioning. Abbreviations: D, digits; E, elbow joint condensation; S, stylopod condensation; Z, zeugopod condensation.

3.2. Probes

Alx3, *Cart1*, *Prx1* and *Prx2*: see Leussink et al. (1995) and Ten Berge et al (1998a,b). *Alx4*: a plasmid containing 0.6 kb *Alx4* cDNA was cut with *Eco*RV and transcribed with T7 polymerase. *Prx3*: a vector containing 220 bp rat *Prx3*A cDNA was cut with *Hin*dIII and transcribed with T3 RNA polymerase.

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