

Gene expression pattern

# Expression pattern of *Irx1* and *Irx2* during mouse digit development

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Received 2 March 2001; received in revised form 26 April 2001; accepted 27 April 2001

## Abstract

*Irx1* and *Irx2* are members of the murine *Iroquois* homeobox (*Irx*) gene family. In this study, we describe the dynamic expression pattern of these genes during limb development with a focus on digit formation. We further present a comparative expression analysis with *Gli* genes (*Gli1*, *Gli2*, *Gli3*). *Gli1*, *Gli2*, and *Gli3* were suggested for candidate regulators of the *Irx* genes. The expression was studied between E11.5 and E14.5 when the digits are being formed. *Irx1* and *Irx2* reproduce the developmental program of the digits in time and space and the *Irx1* provides an early and excellent marker for this process. Our analysis also indicates that the expression of *Irx1*, *Gli1* and *Irx2*, *Gli2* are relative to each other. In contrast, *Gli3* exhibits a different expression pattern. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Digit; *Gli1*; *Gli2*; *Gli3*; *Iroquois*; *Irx1*; *Irx2*; Limb development; Mouse

## 1. Results

The *Irx1* and -2 genes belong to the *Iroquois* homeobox (*Irx*) gene family in mice (Bosse et al., 1997, 2000; Bruneau et al., 2000; Peters et al., 2000).

They exhibit a wide but distinct expression pattern during the nervous system development (Fig. 1A,J,K; Bosse et al., 1997). In this report, we compare the expression patterns of *Irx1* and -2 during the digit development to that of the *Gli* genes (*Gli1*, -2, -3).

At E11.5, all four limb buds are prominent, although the anterior pair is slightly more developmentally advanced than the posterior pair. The extremities are flared out into plates – handplate and footplate (Fig. 2A). By E12.5, the handplate shows the evidence of angular contours at its peripheral margin, and these correspond to the location of the future digits (Fig. 2B). At this time, the footplate is still paddle (Rugh, 1990).

*Irx1* expression is not detected in the developing limb buds before E11.5 (Fig. 1A) where it is detected in the region of the digit primordia 2–4 (Fig. 1B,F). In the remaining digit primordia the expression of *Irx1* appears 1 day later (Fig. 1C,G).

The carpal region is first delineated at about E12.5–E13.5. At the same time, the first digital rays become visible in the handplate by separation of the digital interzones. This

is a critical period for digit formation marked by a transition from mesenchyme to precartilagel (Fig. 2B,C). At E12.5, *Irx1* gene activity is high throughout the developing digits and shows a sharp border at the proximal part (Fig. 1C,G). By E13.5 the limb buds have grown further, while their most distal part is differentiating into early (mesenchymal) digits which are not yet completely distinct (Fig. 2C). During E13.5 the *Irx1* is expressed in the metatarsal cartilage and the cartilage of the phalanges (Figs. 1D,H, 4A).

By E14.5 when all digits of both forelimbs and hindlimbs are splayed out, the *Irx1* expression is maintained in the metatarsal and phalange cartilage (Fig. 1E,I). Therefore, *Irx1* is an excellent marker for digit development.

*Irx2* is also expressed during limb development (Fig. 1J–Q) although this *Iroquois* gene shows an overlapping expression pattern to *Irx1* during the late stages of digit development. Its expression starts later and is different during the initial stages of digit development (Fig. 1L,O). In the region of metatarsal cartilage *Irx2* expression starts at E12.5 but is much weaker than *Irx1* (Fig. 1L,P). Furthermore, the expression exhibits high levels in digits 1 + 5 (asterisks in Fig. 1L,O, 4B (E13.5)). Similar to *Irx1*, the *Irx2* gene shows a border at the proximal part of the developing digits. The expression is less intense and not visible in the region of the phalange cartilage. At E14.5 when the digits become clearly recognizable, *Irx2* is also active in the developing phalanges (Fig. 1N,Q).

In *Drosophila melanogaster*, it was shown that *cubitus interruptus* (*ci*) is a regulator of the *Iroquois* complex (Gómez-Skarmeta and Modolell, 1996). The murine homo-

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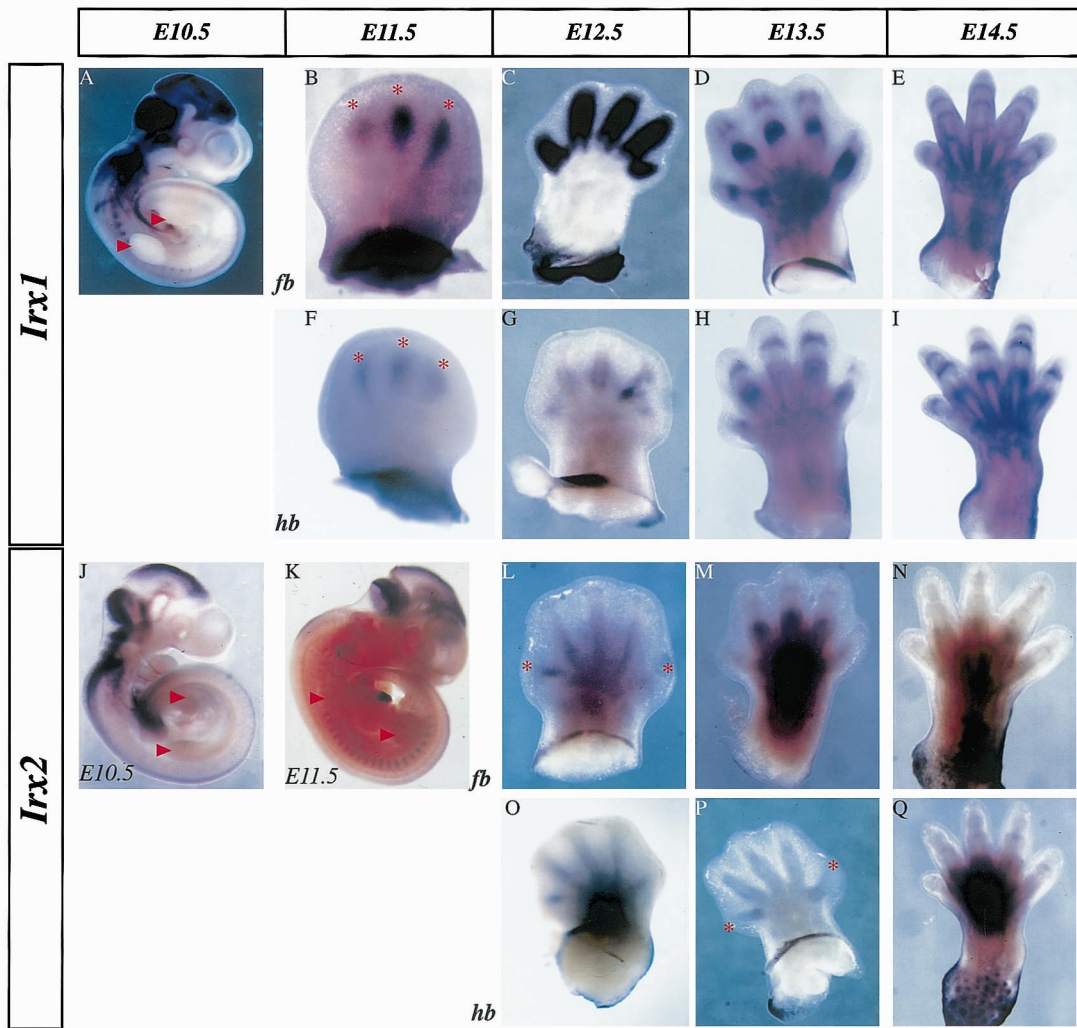


Fig. 1. Expression pattern of *Irx1* and *Irx2* during murine limb development analyzed by whole mount in situ hybridization (A–Q). Arrows ( $\Delta$ ) indicate the regions of the developing limb buds. Asterisks (\*) point to the *Irx* expression in the digits. fb, forelimb; hb, hindlimb.

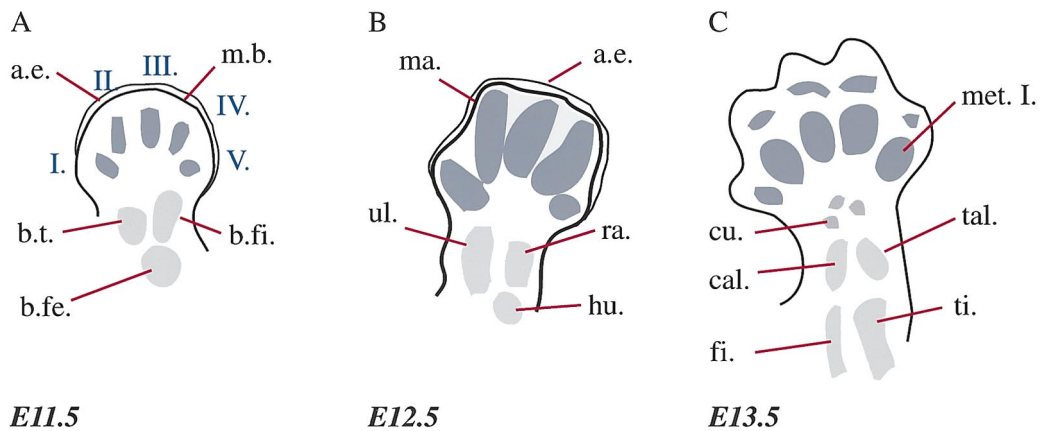


Fig. 2. Scheme of the development of the murine limbs between E11.5 and E13.5 (A–C) (Rugh, 1990; Gruneberg, 1963). a.e., apical ectodermal ridge; b.fi., blastema of fibula; b.fe., blastema of femur; b.t., blastema of tibia; cal., calcaneus; cu., cuboideum; hu., humerus; ma., marginal sinus; m.b., marginal blood sinus; met., metatarsal; ra., radius; tal., talus; ti., tibia; ul., ulna.

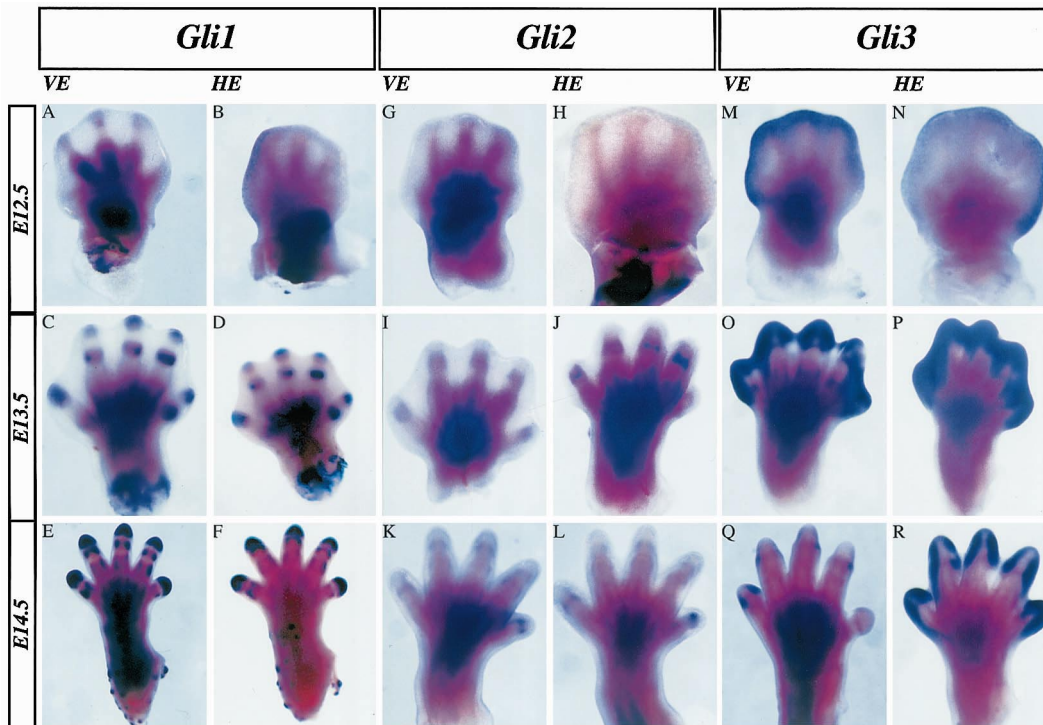


Fig. 3. Expression pattern of *Gli1*, -2, and -3 during murine limb development analyzed by whole mount in situ hybridization (A–R). At E12.5, the *Gli* genes start a second expression phase in the limbs. *Gli1* (A,B) and *Gli2* (G,H) transcripts are found in the condensing mesenchyme, while *Gli3* (M,N) is expressed in the surrounding mesenchyme. *Gli1* is highly expressed in the metatarsal cartilage and the cartilage of the phalanges at E13.5 (C,D). The *Gli1* activity continues to E14.5 (E,F). The expression of *Gli2*, although weak, is similar to *Gli1*. At E12.5, *Gli2* is faintly expressed in the metatarsal cartilage (G,H). At E13.5 and E14.5 *Gli2* is detected in the metatarsal cartilage and the cartilage of the phalanges (I–L). *Gli3*, however, exhibits a different expression pattern and is not detected in the digits during development (O–R). At E12.5 *Gli3* is found in the apical ectodermal ridge and the marginal sinus (P,Q). At E14.5, the expression is still detected in the surrounding mesenchyme (Q,R). VE, forelimb; HE, hindlimb.

logues of *ci* (*Gli1*, -2, -3) are expressed in distinct patterns during limb development (Fig. 3A–R). We therefore compared the expression of the *Irx* genes with the expression of the *Gli* genes during the digit formation. The limb expression of the *Gli* genes starts early at E9.5. At that stage, *Gli2* and -3 are found along the anterior–posterior (A/P) axis of the limb bud (Theil et al., 1999; Büscher and Rütter, 1998).

At E12.5 *Gli1* (Fig. 3A,B) and *Irx1* show a similar expression (Fig. 1C,G). Both genes are found in the metatarsal cartilage and the cartilage of the phalanges at E13.5 and E14.5 (Figs. 3C–F and 4C). The gene activity of *Gli2* is weaker than *Gli1* but is detected in similar domains (Figs. 3G–L and 4D). In contrast, *Gli3* expression pattern is different from that of the other *Gli* genes and also the *Iroquois* (*Irx1*, -2) genes (Figs. 3M–R and 4E).

In summary, the expression domains of *Gli1* and -2 in the

digits seem to correlate with that of the *Irx1* and -2. In contrast, the *Gli3* is not detected in the digits and is restricted to the apical ectodermal ridge.

## 2. Experimental procedures

Embryos (NMRI) were prepared at appropriate stages (day of vaginal plug E0.5). Whole mount in situ hybridization was performed according to standard procedures (Xu and Wilkinson, 1998). Digoxigenin labeled antisense RNA probes were generated.

## Acknowledgements

We are grateful to A. Mansouri and M. Belaousoff for

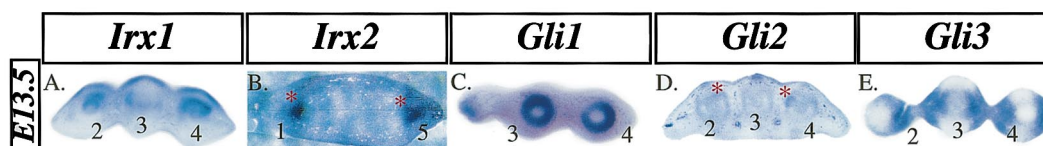


Fig. 4. Cross-section (40  $\mu$ m) of E13.5 murine limbs analyzed by whole mount in situ hybridization (A–E). *Irx1* is at E13.5, expressed in the precartilage of the digits 2–4 (A). The condensed mesenchyme of the digits 1 + 5 are expressing *Irx2* (B). *Gli1* and -2 are expressed in the metatarsal cartilage of the digits 1 – 5 (C,D). In contrast, *Gli3* is expressed in the surrounding mesenchyme (E).

critical reading of the manuscript. We would like to thank A. Joyner for the *Gli1* and -2 probes and U. Rüter for the *Gli3* probe. A.Z. was supported by the Sonderforschungsbereich 271 (SFB271) and M-B.B. is supported by the Human Frontier Science Program RG-0042/1998B.

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