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Gene expression pattern

Fjx1, the murine homologue of the *Drosophila four-jointed* gene, codes for a putative secreted protein expressed in restricted domains of the developing and adult brain

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Abstract

The *Drosophila* gene *four jointed* (*fj*) codes for a secreted or cell surface protein important for growth and differentiation of legs and wings and for proper development of the eyes. Here we report the cloning of the mouse *four-jointed* gene (*fjx1*) and its pattern of expression in the brain during embryogenesis and in the adult. In the neural plate, *fjx1* is expressed in the presumptive forebrain and midbrain, and in rhombomere 4, however a small rostral/medial area of the forebrain primordium is devoid of expression. Expression of *fjx1* in the neural tube can be divided into three phases. (1) In the embryonic brain *fjx1* is expressed in two patches of neuroepithelium: in the midbrain tectum and the telencephalic vesicles. (2) In fetal and early postnatal brain *fjx1* is expressed mainly by the primordia of layered telencephalic structures: cortex (ventricular layer and cortical plate), olfactory bulb (subependymal layer and in the mitral cell layer). In addition expression is observed in the superior colliculus. (3) In the adult, *fjx1* is expressed by neurons evenly distributed in the telencephalon (isocortex, striatum, hippocampus, olfactory bulb, piriform cortex), in the Purkinje cell layer of the cerebellum, and numerous medullary nuclei. In the embryo, strong expression can further be seen in the apical ectodermal ridge of fore- and hindlimbs and in the ectoderm of the branchial arches.

Keywords: Apical ectodermal ridge; Cortex; *four-jointed*; Forebrain development; Olfactory bulb; Regionalization; Telencephalon

1. Introduction

Drosophila four-jointed (*fj*) is expressed in the eye, leg, wing imaginal disks and in the optic lobe (Villano and Katz, 1995; Brodsky and Steller, 1996). The null-mutation of the *Drosophila fj* gene causes reduced and altered growth of the leg and wing along the proximal distal axis (Villano and Katz, 1995; Brodsky and Steller, 1996). Mosaic analysis reveals that this protein works in a non-cell-autonomous manner (Tokunaga and Gerhart, 1976). In accordance, in vitro biochemical studies show that Fj is either a cell surface or a secreted protein (Villano and Katz, 1995). Fj has, therefore, been suggested to be an intercellular signal involved in regulating proximo-distal growth of parts of the legs and wings and in the differentiation of the eyes (Brodsky and Steller, 1996).

2. Results

We have identified a novel mouse gene with high sequence similarity to the *Drosophila fj* gene (Fig. 1A) and we have followed its expression pattern through development. Based on the sequence similarity and the resemblance in expression pattern, we suggest that this cDNA is encoding the homologue of the *Drosophila fj* gene. We therefore, termed this new murine gene *four-jointed x1* (*fjx1*). Partial *fjx1* cDNA clones were isolated from an embryonic day (E) 8.5 mouse embryo cDNA library using a human genomic probe from chromosome 11p13 (Thate et al., 1995). Since the human gene does not contain an intron

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(M. Gessler et al., in preparation), complete mouse genomic clones were isolated in parallel and sequenced. The murine sequence has an open reading frame encoding a polypeptide of 437 amino acids. The calculated relative molecular mass of the conceptual translation product is 48 912 Da, and its isoelectric point would be 10.55. Comparison of the predicted amino acids sequence with the currently available database entries revealed significant homology to the *Drosophila* Fj protein (50% similarity, 42% identity, see Fig. 1). In addition to sequence similarity, Fj and Fjx1 share structural motives (Fig. 1A); a putative transmembrane domain, and a signal sequence cleavage site (von Heijne, 1986) are located in the N-terminal end of both proteins. In the C-terminal region of both proteins consensus sites for asparagine linked glycosylation are present. In addition, a consensus sequence for alpha amidation, a common modification in proteolytically processed neuropeptides (Eipper et al., 1992), is predicted in Fj and Fjx1. Finally, the predicted topology of both proteins implies that the C-terminal region is processed in the endoplasmic reticulum and secreted (Hartmann et al., 1989). This is in agreement with in vitro experimental evidence showing that the *Drosophila* Fj is glycosylated and secreted (Villano and Katz, 1995).

In order to determine the expression pattern of *fjx1*, polyA RNA from embryos of E7–17 was examined by Northern blot analysis (Fig. 1B). A single *fjx1* transcript of 2.8 kb was detected from E11 onward. A transcript of the same size was also detected in the adult brain (Fig. 1B). A weaker signal corresponding to 1.7 kb is detected at E7, but not during the later stages of development.

2.1. Embryonic pattern of expression

In the neural plate (E8.5; Fig. 1C,D) *fj*x is expressed in a large rostral area which corresponds to the presumptive forebrain and midbrain (Fig. 1C), although excluding a rostral/medial region (asterisk in Fig. 1D). In addition, *fjx1* is most probably expressed in rhombomere 4 (Fig. 1C; identified following morphological criteria). At E9.5 (Fig. 1E,F), *fjx1* expression is observed in the limb buds and in the ectoderm of the first branchial arches (arrow head in Fig. 1F). In the brain, expression is detected in two distinct regions, the dorsal mesencephalon (tectum) and prosencephalon (presumptive isocortex). The expression in the tectum and the cortex persists at E10.5. (Fig. 1G,H) and E11.5 (Fig. 1I,J). In the cortex, *fjx1* is expressed specifically by a restricted dorsolateral patch of the neuroepithelium. Strong expression is observed in the ectoderm of the branchial arch and the oral ectoderm (Fig. 1K). In the limbs *fjx1* is expressed in the AER (Fig. 1I,J).

2.2. Fetal and early postnatal pattern of expression (Fig. 2A–H)

During the histogenetic period of brain development *fjx1* is highly expressed by a number of layered structures, particularly in the telencephalon (Fig. 2A–D), but also in the tectum/superior colliculus and the cerebellum (Fig. 2E–H).

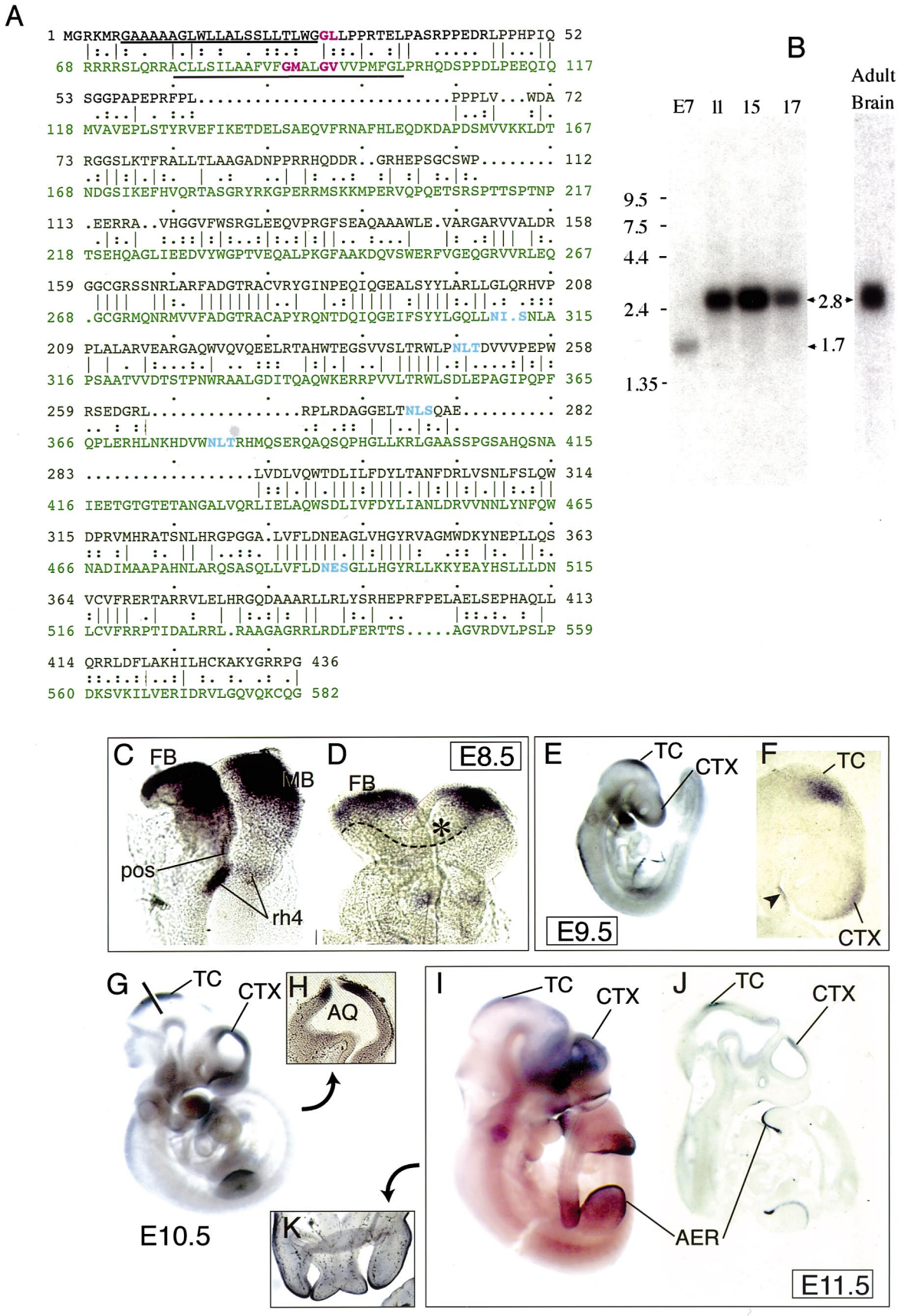
2.3. Adult pattern of expression

In the adult (postnatal day 30), *fjx1* is strongly expressed by numerous cells evenly distributed throughout the telencephalon (Fig. 2I). Expression seems particularly intense in the piriform cortex, the hippocampus and the olfactory bulb. In the diencephalon, only the dorsal thalamus expresses *fjx1*, although weakly. The Purkinje layer of the cerebellum, as well as numerous medullary nuclei also express our gene.

3. Methods

An E8.5 day mouse embryo cDNA library (a gift of B. Hogan) and a mouse genomic library (strain 129SVJ) were screened with a 1.7 kb NotI–BamHI fragment from the human NE3 locus (D11S3892) using standard conditions except for a reduced hybridization and washing temperature of 55°C (Sambrook et al., 1989). The isolation of the human genomic probe was described in Thate et al., 1995. The region homologous to *fjx1* was sequenced and a 2088 bp

Fig. 1. (A) Comparison between the predicted amino acid sequences of the *four jointed* genes in mouse and *Drosophila*. The sequence of mouse Fjx1 (black) as compared with *Drosophila* Fj (green) reveals high sequence homology (50% similarity, 42% identity; comparison done by the Bestfit GCG program). A predicted (Kyte and Doolittle, 1982) hydrophobic cluster is located between AA 7–27 in Fjx and between AA 77–103 in Fj (underlined). A potential signal sequence cleavage site (von Heijne, 1986) is predicted in both proteins (red). In both proteins putative glycosylation sites (blue) are present. Alpha amidation consensus sequences (XGR/K,R/K) are predicted between residues 1–4 and 431–433 in Fjx1 and between residues 535 and 538 in Fj. (B) Expression of *fjx1* mRNA during embryogenesis and in the adult brain. PolyA RNA from embryos of E7,11,15,17 (left) and 20 μg of total RNA from adult brain (right) were examined by Northern blot analysis using a 1 kb probe encompassing most of the transcribed region. The size of the marker and the labeled bands is indicated in kb. (C–K) Pattern of expression of *fjx1* during early development shown by whole mount in situ hybridization. (C) Dorsal and (D) head-on views of labeled neural plates (E8.5). The asterisk in (D) shows the non-labeled medial/rostral area in the presumptive forebrain. The dotted line shows the profile of the rostral end of the neural plate. (E) E9.5 embryo treated with antisense probe. (F) Sagittal section of E9.5 embryo, showing a detail of the labeled neuroepithelial regions. Arrowhead points at the epibranchial ectoderm. (G) Expression of *fjx1* at E10.5. The thick line in the tectum shows the approximate plane of section of (H). (H) Transverse section of the embryo in (G) showing the labeled tectal neuroepithelium. (I) Expression of *fjx1* at E11.5. (J) Sagittal section through the embryo shown in (I). (K) Transverse section through the face of a littermate of the embryo shown in (I and J), demonstrating the labeling in the epibranchial and the oral ectoderm. Abbreviations: AER, apical ectodermal ridge; AQ, aqueduct; CTRL, control; CTX, cortex; FB, forebrain; MB, midbrain; pos, preotic sulcus; rh4, fourth rhombomere; TC, tectum.


A
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E8.5
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E9.5
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Fig. 1.

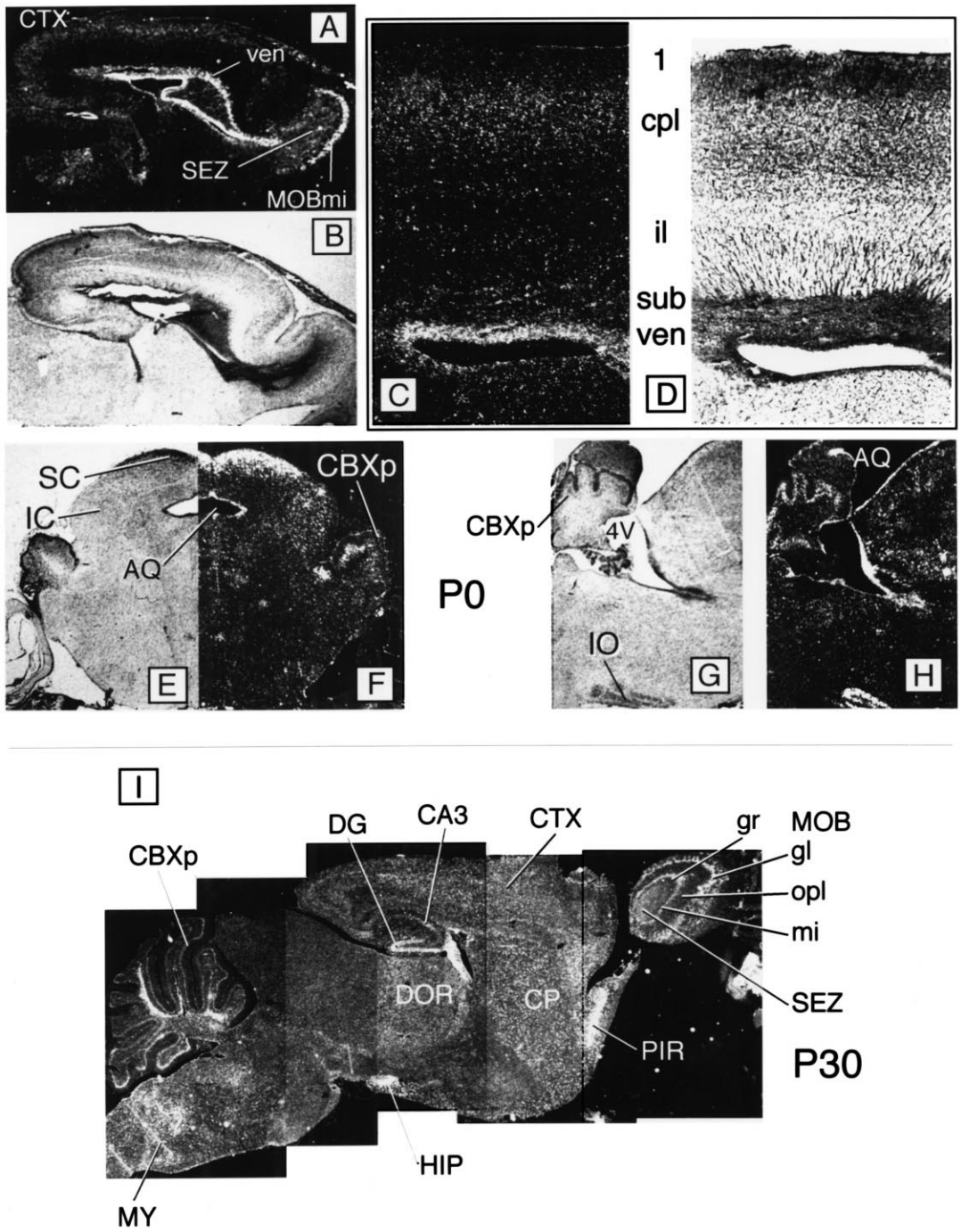

Fig. 2. Expression of *fjx1* in fetal and adult brains demonstrated by in situ hybridization. (A,B) Sagittal sections through a mouse P0 brain (A, dark field; B, bright field) showing *fjx1* expression in the cortex and olfactory bulb. (C,D) High-magnification photograph of the P0 cortex (C, dark field; D, bright field) showing *fjx1* expression in the ventricular layer and in the cortical plate of the developing isocortex. (E,F) Transverse section (E, bright field; F, dark field) through the P0 brainstem showing expression in the superior colliculus and Purkinje cell layer of the cerebellum. (G,H) Sagittal section through the P0 brainstem (G, bright field; H, dark field) showing expression in the ependymal layer of aqueduct and fourth ventricle, as well as in the inferior olive and the cerebellum. (I) Sagittal section through a P30 brain. Notice the strong expression in the telencephalon especially in the dentate gyrus, in layer 2 of the Piriform cortex and in the olfactory bulb. *Fjx1* expression in main olfactory bulb is observed in the subependymal zone, the mitral cell layer and in the glomerular layer but not in the granular layer or the outer plexiform layer. Abbreviations: 1, isocortical layer 1; 4V, fourth ventricle; AQ, aqueduct; CA3, region 3 of Ammon's horn (hippocampus); CTX, cortex; CBXp, Purkinje cell layer of cerebellar cortex; CP, caudate putamen; cpl, cortical plate; DG, dentate gyrus (hippocampus); DOR, dorsal thalamus; GB, globus pallidus; HIP, hippocampus; IC, inferior colliculus; il, intermediate layer; IO, inferior olivary nucleus; gl, glomerular layer; gr, granular layer; mi, mitral cell layer; opl, outer plexiform layer; MOB, main olfactory bulb; MY, medulla (myelencephalon); PIR, Piriform cortex; SC, superior colliculus; SEZ, subependymal zone; sub, subventricular layer; ven, ventricular layer (neuroepithelium).

segment spanning the entire coding region and the flanking untranslated regions (EMBL accession number AJ009634).

A ^{32}P-labeled probe (SacII–PflMI, 1 kb fragment) was hybridized to a commercially available mouse embryo Northern blot membrane (Clontech 7763-1) and to a membrane on which 20 μg total RNA from adult brain has been blotted. In situ hybridization experiments on whole-mounts or tissue sections were performed as previously described (Simmons et al., 1989; Nieto et al., 1996) using a 1.6 kb probe (SacII–EcoRI fragment). Identification and naming of brain structures was done after Alvarez-Bolado and Swanson (1996).

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