The rodent Four-jointed ortholog Fjx1 regulates dendrite extension

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Abstract

The extrinsic and intrinsic factors that regulate the size and complexity of dendritic arborizations are still poorly understood. Here we identify Fjx1, the rodent ortholog of the Drosophila planar cell polarity (PCP) protein Four-jointed (Fj), as a new inhibitory factor that regulates dendrite extension. The Drosophila gene four-jointed (fj) has been suggested to provide directional information in wing discs, but the mechanism how it acts is only poorly understood and the function of its mammalian homolog Fjx1 remains to be investigated. We analyzed the phenotype of a null mutation for mouse Fjx1. Homozygous Fjx1 mutants show an abnormal morphology of dendritic arbors in the hippocampus. In cultured hippocampal neurons from Fjx1 mutant mice, loss of Fjx1 resulted in an increase in dendrite extension and branching. Addition of Fjx1 to cultures of dissociated hippocampal neurons had the opposite effect and reduced the length of dendrites and decreased dendritic branching. Rescue experiments with cultured neurons showed that Fjx1 can act both cell-autonomously and non-autonomously. Our results identify Fjx1 as a new inhibitory factor that regulates dendrite extension.

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Introduction

The shape and complexity of dendritic arborizations is an important determinant of their function to receive and process information. Despite recent progress in identifying intrinsic and extrinsic factors that regulate dendrite architecture (Jan and Jan, 2003; Whitford et al., 2002a), the signaling pathways that determine the pattern of dendritic arborizations are not well understood. Genetic approaches and in vitro systems allowed the identification of contact-dependent and secreted signals that regulate the development of dendritic arbors. These include signals that stimulate dendrite growth or act as chemotropic guidance cues like Sema3A, Slit1, neurotrophins, BMPs, or Wnt7b (Polleux et al., 2000; Rosso et al., 2005; Whitford et al., 2002a,b). In addition, proteins were identified that mediate contact-dependent homotypic repulsion and limit the extension of dendrites (Whitford et al., 2002a).

A screen for genes that determine the size and shape of dendritic fields in Drosophila led to the identification of Flamingo (Fmi) as a signal that mediates homotypic repulsion between dendrites (Gao et al., 1999, 2000; Grueber et al., 2002; Reuter et al., 2003). Fmi encodes an atypical cadherin with a large extracellular domain and seven transmembrane segments (Usui et al., 1999). Fmi and its three mammalian homologs (Celsr1–3) are core components of the PCP pathway that determines the polarity of cells in the plane of an epithelium, e.g. in the Drosophila eye and wing imaginal discs (Curtin et al., 2003; Fanto and McNeill, 2004; Klein and Mlodzik, 2005). A set of core components is required for PCP in multiple tissues and is highly conserved between species (Seifert and Mlodzik, 2007; Wang and Nathans, 2007; Zallen, 2007). The available evidence suggests that the atypical cadherins Dachsous (Ds) and Fat (Ft) control PCP upstream of the integral membrane protein Frizzled (Fz) and the cytosolic protein Dishevelled (Dsh) to transmit the positional information for the orientation of wing hairs (Klein and Mlodzik, 2005; Tanoue and Takeichi, 2005). In vertebrates, the PCP pathway is required for convergent extension during gastrulation and the development of the sensory epithelium in...
the inner ear. In addition, Celsr2 and -3 have been implicated in neuronal differentiation (Shima et al., 2007; Takeichi, 2007; Wada et al., 2006). Celsr2 is involved in the development of dendritic trees of cortical and cerebellar neurons and is thought to maintain the size and complexity of dendritic arbors by homophilic interactions (Shima et al., 2004). Deletion of Celsr3 in mice disrupts the formation of major axon fascicles (Tissir et al., 2005).

Genetic experiments suggest that the type II transmembrane protein Four-jointed (Fj) acts upstream of Ds and Ft (Amonlirdviman et al., 2005; Ma et al., 2003). Fj, Ds, and Ft are thought to provide global directional information in the wing disc that aligns the activity of Fz/Dsh in different cells (Amonlirdviman et al., 2005; Ma et al., 2003). The role of Fj is not as well understood as that of other PCP proteins. Proteolytic cleavage of Fj in its transmembrane domain releases the extracellular C-terminal fragment (Buckles et al., 2001; Strutt et al., 2004). Both, a function as a secreted ligand and a role in the maturation of other proteins in the Golgi have been proposed (Buckles et al., 2001; Strutt et al., 2004). It was reported recently that Fj can be found mainly in the Golgi of Drosophila S2 cells and imaginal discs (Strutt et al., 2004). Cleavage and secretion apparently are not required for its function since a Fj protein fused to a Golgi localization signal has increased activity compared to the wild type protein (Strutt et al., 2004). However, the mammalian Fj ortholog Fjx1 (Ashery-Padan et al., 1999) is secreted efficiently when expressed in cell lines (Rock et al., 2005a).

Mouse Fjx1 is expressed in the developing brain, like the mammalian orthologs of Fmi, Ft, and Ds (Ashery-Padan et al., 1999; Rock et al., 2005b; Shima et al., 2002; Tissir et al., 2002). An AP fusion protein of Fjx1 revealed the presence of Fjx1 binding sites in the cortex and hippocampus, supporting the possibility that Fjx1 acts as a ligand for an unknown receptor (Rock et al., 2005a). Here we analyzed the phenotype of Fjx1 knockout mice and show that Fjx1 is required for the normal development of dendritic arbors in the hippocampus. Our results identify Fjx1 as a new signal that directs the development of dendrites.

Materials and methods

Fjx1 knockout mice

The Fjx gene is encoded by a single exon that contains the entire coding sequence. The exon was replaced by the LacZ gene followed by a PGK-neo resistance cassette. A 4.1 kb EcoRI/Smal fragment containing the promoter region and 56 bp of 5‘ UTR was cloned in front of a lacZ/PGK-neo cassette in pHM2. A 1.7 kb BamH/HindIII fragment containing 632 bp of the 3‘ UTR was inserted downstream and the entire insert was then transferred into pPNT (a gift of R.C. Mulligan) to add the HSV-tk negative selection marker. 129/SVJ ES cells were electroporated with the linearized plasmid. Positive clones were identified by Southern blot using a 1.3 kb BamH/HindIII fragment containing the promoter and 56 bp of 5‘ UTR. One positive clone was injected into C57/B6 blastocysts. Germ line transmission was confirmed by Southern blot and PCR. Genotyping by PCR was done using the primers 4J5A (AGGGC TGTCT TCTCT GCCACG), 4J3A (TCCCA AAGAG CO2. After neurons attached to the substrate (around 4 h after plating) the culture was continued in the presence of 3 μM ARAc, 0.5 mM glutamine and 100 U/ml penicillin/streptomycin (Invitrogen). Neurons were fixed with methanol/acetone (1:1) for 20 min at −20 °C. After washing with neurobasal medium, the culture was continued in the presence of 3 μM ARAc (Sigma). At 7 d.i.v., neurons were fixed in 4% paraformaldehyde and 15% sucrose in phosphate-buffered saline (PBS) for 20 min at room temperature and processed for immunohistochemistry. Expression vectors containing the complete coding sequence of mouse Fjx1 or of Fjx1 fused to alkaline phosphatase have been described before (Rock et al., 2005a). pEGFP-N1 (Clontech) was used as control vector.

Immunofluorescence

The mouse monoclonal Tau-1 and anti-MAP2 antibodies were obtained from Chemicon. Polyclonal rabbit anti-Fjx1 was described before (Rock et al., 2005a). Alexa-594, Alexa-488, and Alexa-350 conjugated secondary antibodies were obtained from Molecular Probes. Neuronal morphology was analyzed using a Zeiss Axiophot microscope equipped with an Orca CCD camera (Hamamatsu) and the WASABI software (Hamamatsu). Image tool, and Adobe Photoshop. Neurites that are at least twice as long as the other processes and showed Tau-1 immunoreactivity in their distal segments were counted as axons. MAP2-positive neurites longer than the diameter of the cell soma were counted as dendrites.

Generation of recombinant Fjx1 protein

A stable HEK293 cell line expressing a fusion protein of Fjx1 and placental alkaline phosphatase (AP) (Flanagan and Leder, 1990) was used to obtain recombinant Fjx1 (Rock et al., 2005a). 293-fjx1-AP cells were maintained in 0.6 μm gold particles (BioRad) were coated with 1,1′-diocadecyl-3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI, Molecular Probes) based on published procedures (Gan et al., 2006). Briefly, 15 μg gold particles were mixed with 5 μg Dil dissolved in 500 μl methylene chloride. Gold particles were coated with Dil by evaporation of methylene chloride, resuspended in 3 ml distilled water and 0.75 μl polyvinylpyrrolidone (20 mg/ml) was added to the sonicated solution. The suspension was used to coat Tefzel tubing (Biorad) according to the manufacturer’s recommendation. P15 Fjx1 mouse brains were fixed with 4% PFA in PBS overnight at 4 °C. The hippocampus was dissected and Dil-coated gold particles were delivered using the Helios Gene Gun System (Biorad) with a pressure of 160–180 psi. A membrane filter with a 3-μm pore size and 8.0×109 pores/cm² (Falcon) was inserted between the Gene Gun and the tissue (Gan et al., 2000). The hippocampus was incubated for 5–7 days in PBS at 30 °C. 100 μm coronal sections were prepared using a vibratome (Leica) and analyzed by confocal microscopy (Zeiss Axiovert 200 M) and the Zeiss AIM LSM510 Image Browser software. Care was taken that the analyzed neurons were fully contained within the z stack collected.
DMEM containing 10% fetal calf serum (Biochrom), 100 U/ml penicillin/streptomycin, and 0.8 mg/ml G418 (Invitrogen) at 37 °C and 5% CO₂. Conditioned medium was obtained by incubating confluent cells with serum-free DMEM for 2 days. The supernatant was cleared and concentrated 15-fold by ultrafiltration using Amicon Ultra Centrifugal Filter Devices (Millipore). As control, medium was conditioned by untransfected HEK 293T cells or by HEK 293T cells that were transfected with an expression vector for secreted AP (Flanagan and Leder, 1990) by calcium phosphate coprecipitation. No difference in the number of neurites, dendrite length, or number of branches per dendrite was observed when untreated cells and cells treated with control medium were compared (Fig. 1, and data not shown). To ensure an equal concentration of recombinant Fjx1-AP and AP, an assay for alkaline phosphatase (Sigma) was used according to the manufacturer’s instructions. Medium containing recombinant protein was added to neuronal cultures at 6 d.i.v. for 24 h.

**Results**

_Fjx1 is required for dendrite development_

_Fjx1_ knockout mice were generated by deleting the complete coding sequence that is contained within a single exon (Fig. 1A). Homozygous _Fjx1_ mutant mice were healthy and fertile and showed no overt morphological or behavioral defects (Fig. 1B, and data not shown). _Fjx1_ is highly expressed in the cerebellum, cortex, hippocampus, and olfactory bulb (Ashery-Padan et al., 1999; Rock et al., 2005b). However, the structure of the cortex and hippocampus was normal (Fig. 2A, and data not shown). The expression pattern of _Fjx1_ mRNA and the distribution of _Fjx1_ binding sites in the adult brain are largely complementary, especially in the hippocampus (Rock et al., 2005a,b).

In the hippocampus, _Fjx1_ binding sites are detectable mainly in regions that contain dendrites. To analyze the morphology of hippocampal neurons, _Fjx1_ knockout mice were crossed to the transgenic line _B6.Cg-TgN(Thy1-YFPH)2Jrs_, which expresses YFP in a subset of neurons, allowing to analyze their morphology (Feng et al., 2000). In homozygous _Fjx1_ mutants, the dendritic trees of pyramidal neurons in the CA1 region of the hippocampus appeared to be less complex than in wild type mice (Fig. 2A). Since the available antiserum did not allow the detection of endogenous _Fjx1_ in Western blots, cultured neurons were analyzed by immunofluorescence to confirm
that no Fjx1 protein was produced in homozygous mutants. Staining of hippocampal neurons isolated from E18 wild type mouse embryos with an antibody specific for Fjx1 (Rock et al., 2005a) revealed a punctate pattern in the cell body and in neurites (Fig. 2B). Fjx1 immunoreactivity was strongly diminished in neurons derived from heterozygous knockouts and reduced to background levels in Fjx1−/− neurons, confirming the absence of the protein in homozygous Fjx1 mutants.

Defects of dendritic arbor formation in the hippocampus

To analyze the phenotype of Fjx1 mutants in detail, the morphology of single neurons was visualized in the hippocampus of P15 mice by DiI labeling. The total length of dendrites formed by granule cells in the DG showed an increase by 20% in Fjx1 mutants (Fig. 3; Fjx1+/−: 197±7 μm; n=70; 8 animals) compared to wild type hippocampus (164±7 μm; n=56; 7 animals), suggesting the absence of an inhibitory signal. The complexity of the dendritic tree as determined by the number of first, second, and third order branches per cell was normal in Fjx1 mutants (n=15 neurons each for wild type and Fjx1−/−).

The analysis of dendritic arbors from pyramidal neurons in the CA1 region of Fjx1−/− mice revealed a slightly different phenotype (Fig. 4). The length of the apical dendrite was not significantly different from wild type mice (Figs. 4A–C; wild type: 407±30 μm, n=28 neurons from 8 animals; Fjx1−/−: 401±21 μm, n=19, 8 animals). However, the complexity of the apical dendrites from neurons lacking Fjx1 was clearly reduced. The number of first, second, and third order branches per neuron was significantly lower in Fjx1−/− animals compared to wild type mice (Figs. 4A, B, D; wild type: 5.3±0.4 first-order branches, 4.2±0.4 second-order branches, 0.8±0.2 third-order branches, 10.3±1.0 dendritic branches per cell in total, n=28; Fjx1−/−: 2.9±0.4 first-order, 1.4±0.3 second-order, 0.2±0.1 third-order, 4.5±0.8 total, n=19). Thus, mutation of Fjx1 results in defects of dendritic arbor development in the hippocampus that differ between the DG and the CA1 region.

To investigate the role of Fjx1 for dendrite development in more detail, we used primary cultures of dissociated neurons from the embryonic hippocampus of wild type and mutant embryos. These cultures contain neurons both from the CA1 region and from the dentate gyrus. The morphology of dissociated hippocampal neurons from E18 wild type, Fjx1+/−, and Fjx1−/− embryos was analyzed at 3 (Fig. 5) and 7 (Fig. 6) days in vitro (d.i.v.) by staining with an anti-MAP2 antibody as a marker for dendrites (Schwamborn et al., 2006). Cultures of dissociated hippocampal neurons from rat or mouse embryos are a well-established model system for neuronal differentiation (Dotti et al., 1988). In these cultures, dendrite growth starts at 3–4 d.i.v. (stage 4; Dotti et al., 1988; Schwamborn et al., 2006). A significant difference in dendrite length was detectable at 3 d.i.v. (Fig. 5A). The length of dendrites was increased by 56% in neurons prepared from Fjx1−/− embryos (wild type: 25±1 μm, n=71; Fjx1−/−: 39±2 μm, n=71). No difference in the extension of axons was observed (Fig. 5C; wild type: 142±6 μm, n=67; Fjx1−/−: 141±7 μm, n=61; 3 experiments). At 7 d.i.v., dendrite length was increased by 50% in Fjx1−/− and by 88% in Fjx1−/− neurons (Figs. 6A, B; wild type: 34±1 μm, n=289; Fjx1+/−...
51± 1 μm, n=178; Fjx1<sup>−/−</sup>: 64±1 μm, n=142; 4 experiments). In addition, the number of dendritic branches per cell (Fig. 6C) increased from 1.2±0.1 in wild type (n=80) or 1.4±0.1 in Fjx1<sup>−/−</sup> neurons (n=62) to 1.8±0.2 in homozygous mutants (n=70; 4 experiments). The number of axons and dendrites per cell did not differ between wild type and mutant neurons at both stages examined (Figs. 5C, 6D).

**Rescue of the mutant phenotype by Fjx1**

Addition of different amounts of recombinant Fjx1 (Rock et al., 2005a) to cultures of hippocampal neurons at 6 d.i.v. induced a dose-dependent decrease in the length of dendrites and the number of dendritic branches (Supplementary Fig. 1). Thus, exogenous Fjx1 had an inhibitory effect on dendrite growth, opposite to that of a loss of Fjx1. To test if secreted recombinant Fjx1 is able to rescue the phenotype of neurons from Fjx1<sup>−/−</sup> mice, recombinant Fjx1 was added to cultures derived from E18 Fjx1<sup>−/−</sup> embryos and the morphology of dendritic arbors analyzed at 7 d.i.v. (Figs. 6B–D). Exogenous Fjx1 reduced the length of dendrites in cultures from Fjx1<sup>−/−</sup> mice to wild type levels (37±1 μm; n=123). In addition, the number of dendritic branches was significantly reduced (0.9±0.1; n=65). The number of axons or dendrites per cell was not changed. Thus, Fjx1 can regulate dendrite growth both cell-autonomously and as a ligand when added to the cultures.

**Discussion**

The phenotype of mutant mice and the effects of recombinant Fjx1 on cultured neurons identify Fjx1 as a new inhibitory factor that regulates dendrite growth. A mouse mutant for Fjx1 displayed defects in dendrite growth and branching both in vivo and in neuronal cultures. While the overall organization of the hippocampus was normal in Fjx1<sup>−/−</sup> mice, labeling of individual neurons revealed defects in the formation of dendrites. In homozygous mutants, we observed an increase in dendrite length of granule cells in the dentate gyrus and a reduction in the complexity of dendritic arborizations of pyramidal neurons in the CA1 region. In cultured hippocampal neurons from mutant mice, an increase in dendrite length and branching was observed, indicating the absence of an inhibitory signal. Interestingly, heterozygous Fjx1<sup>−/−</sup> mutants displayed a phenotype intermediate between that of wild type mice and homozygous mutants, consistent with the dose-dependent effects of exogenous Fjx1 on dissociated hippocampal neurons.
While the complexity of dendritic arbors was reduced, a significant difference in dendrite length was not detectable in the CA1 region of mutant mice. The less pronounced phenotype of CA1 pyramidal neurons compared to cultured neurons prepared from embryonic hippocampus may reflect the reduced complexity of the in vitro environment. The absence of region-specific signals that direct the development of dendrites may render neurons more sensitive to the absence of regulatory cues. A difference between the phenotypes of neurons analyzed in primary cultures and in vivo was observed for example also in the mouse mutants for the p190 RhoGAP (Brouns et al., 2001). Alternatively, the reduction in dendrite complexity could result from cell-contact dependent effects that are not reproduced in culture. Independent of the underlying mechanism, the phenotype of Fjx1−/− neurons indicates that restraining the growth of dendrites is only one aspect of Fjx1 function.

Both a function as a ligand for an unknown receptor and as a modulator that regulates the activity of other proteins in the Golgi has been suggested for Fj. The transmembrane segment of Fj contains predicted protease cleavage sites and the release

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Fig. 4. Fjx1 reduces the complexity of dendritic arbors formed by CA1 pyramidal neurons. (A) Single pyramidal neurons from the CA1 region of P15 wild type or Fjx1−/− mice were labeled with Dil and coronal sections (100 μm) analyzed by confocal microscopy. (B) Camera lucida drawings of representative Dil-labelled neurons are shown. (C) The length of apical dendrites from CA1 pyramidal neurons (mean±S.E.M.) of wild type (white bar) or Fjx1−/− animals (black bar) is shown. (D) The number of first, second, and third order branches and the total number of branches per cell is shown (***P<0.001 compared to wild type; *P<0.02 compared to wild type). The scale bar is 50 μm.
of the C-terminus has been demonstrated in vitro and in vivo (Buckles et al., 2001). Although its C-terminal part is secreted, Drosophila Fj is most active when located in the Golgi. These results are consistent with the conclusion that Fj modulates the activity of other unknown secreted or membrane-bound signaling molecules by mediating their post-translational

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Fig. 5. Dendrite length and branching are increased in Fjx1-deficient neurons at 3 d.i.v. (A–C) Hippocampal neurons from wild type (white bars) and Fjx1−/− mice (black bars) were fixed at 3 d.i.v. and stained with anti-MAP2 and Tau-1 antibodies. The length of dendrites (A; means±S.E.M.), the number of dendritic branches per cell (B), and the number of axons and dendrites per cell (C) were determined.

Fig. 6. Dendrite length and branching are increased in Fjx1-deficient neurons at 7 d.i.v. (A–D) Hippocampal neurons from wild type (wt), Fjx1+/−, and Fjx1−/− mice were fixed at 7 d.i.v. and stained with anti-MAP2 (green) and Tau-1 (red) antibodies. The length of dendrites (B), the number of dendritic branches per cell (C), and the number of axons and dendrites per cell (D) were determined for neurons from wild type (white bars), Fjx1+/− (grey bars), or Fjx1−/− mice (black bars). Neurons from Fjx1−/− mice were treated at 6 d.i.v. with 200 μl Fjx1 containing medium (hatched bars), fixed on 7 d.i.v. and the length of dendrites (B), the number of dendritic branches per cell (C), and the number of axons and dendrites per cell (D) were determined (**P<0.001 compared to wild type or between the genotypes indicated by brackets; *P<0.006 compared to wild type; 3 experiments). The scale bar is 50 μm.
modification (Strutt et al., 2004). By contrast, vertebrate Fjx1 is processed efficiently and secreted as an active protein when expressed in a stable cell line (Rock et al., 2005a) as demonstrated by its effects on cultured neurons.

Our results provide evidence both for a cell-autonomous and a non-autonomous function of Fjx1. Binding sites for Fjx1 can be detected mainly in regions of the hippocampus that contain dendrites. The distribution of Fjx1 binding sites in the adult brain are largely complementary to the expression pattern of Fjx1 mRNA (Rock et al., 2005a,b). Both, application of exogenous Fjx1 and transfection with a Fjx1 expression vector were able to rescue the loss of Fjx1, at least with respect to dendrite extension. Whereas transfection of Fjx1−/− neurons with an expression vector for Fjx1 did not result in a significant change in branching, exogenous Fjx1 reduced the number of branches formed by Fjx1 deficient neurons to wild type levels. Thus, Fjx1 may act through different mechanisms on dendrite growth and branching. These results are consistent with the possibility that Fjx1 acts as a paracrine or autocrine signaling factor. Alternatively, Fjx1 might modulate the activity of other proteins on the cell surface of neurons or intracellularly after endocytosis. Future experiments will have to address the molecular mechanism of its function in neurons. Independent of the mechanism, however, our results show that Fjx1 does not require cell contact to regulate dendrite growth and can act as a secreted signal.

In Drosophila, homotypic repulsion between like dendrites limits the size of dendritic fields formed by a specific type of PNS neurons (Gao et al., 1999, 2000; Grueber et al., 2002; Reuter et al., 2003; Whitford et al., 2002a). The repulsion between the dendrites of these neurons is mediated by Fmi. Another example for a signal mediating the repulsion between dendrites is Notch that restricts the growth of dendrites in cultures of cortical pyramidal neurons, an effect that requires nuclear signaling (Franklin et al., 1999; Redmond et al., 2000; Sestan et al., 1999). Notch proteins also stimulate Drosophila Fj and mouse Fjx1 expression (Buckles et al., 2001; Papayannopoulos et al., 1998; Rock et al., 2005a; Zeidler et al., 1999).

Fig. 7. Transfection with a Fjx1 vector rescues the loss of Fjx1. (A–D) Hippocampal neurons from Fjx1−/− mice were transfected at 3 d.i.v. with an expression vector for EGFP (green) or EGFP and Fjx1, fixed on 7 d.i.v., and stained with an anti-MAP2 (red) antibody. The length of dendrites (B; means±S.E.M.), the number of dendritic branches per cell (C), and the number of axons and dendrites per cell (D) are shown for neurons expressing EGFP (white bars), EGFP and Fjx1 (black bars), or untransfected neurons from cultures transfected with vectors for EGFP and Fjx1 (grey bars) (**P<0.001 compared to EGFP; 3 experiments). The scale bar is 50 μm.
These results suggest that Notch may act by inducing the expression of molecules that inhibit neurite growth such as Fjx1. The availability of an in vitro system will allow to study the signaling pathways that mediate the effects of Fjx1 and possible interactions with Notch and Wnt pathways.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2007.09.054.

References


of facial motor neurons in the developing zebrafish hindbrain. Development 133, 4749–4759.