Development 115, 737-744 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

Expression pattern of *Motch*, a mouse homolog of *Drosophila Notch*, suggests an important role in early postimplantation mouse development

FRANCISCO FRANCO DEL AMO[1], DAVID E. SMITH[1], PAMELA J. SWIATEK[1], MAUREEN GENDRON-MAGUIRE[1], RALPH J. GREENSPAN[2], ANDREW P. MCMAHON[1] and THOMAS GRIDLEY[1,*]

[1]*Department of Cell and Developmental Biology* and [2]*Department of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110, USA*

*Corresponding author

Summary

The *Notch* gene of *Drosophila* encodes a large transmembrane protein involved in cell-cell interactions and cell fate decisions in the *Drosophila* embryo. To determine if a gene homologous to *Drosophila Notch* plays a role in early mouse development, we screened a mouse embryo cDNA library with probes from the *Xenopus Notch* homolog, *Xotch*. A partial cDNA clone encoding the mouse *Notch* homolog, which we have termed *Motch*, was used to analyze expression of the *Motch* gene. *Motch* transcripts were detected in a wide variety of adult tissues, which included derivatives of all three germ layers. Differentiation of P19 embryonal carcinoma cells into neuronal cell types resulted in increased expression of *Motch* RNA. In the postimplantation mouse embryo *Motch* transcripts were first detected in mesoderm at 7.5 days post coitum (dpc). By 8.5 dpc, transcript levels were highest in presomitic mesoderm, mesenchyme and endothelial cells, while much lower levels were detected in neuroepithelium. In contrast, at 9.5 dpc, neuroepithelium was a major site of *Motch* expression. Transcripts were also abundant in cell types derived from neural crest. These data suggest that the *Motch* gene plays multiple roles in patterning and differentiation of the early postimplantation mouse embryo.

Key words: *Notch*, mouse development, neurogenesis, P19 cells, *Motch*, *Xotch*.

Introduction

The differentiation of the nervous system is a key event in the early development of many organisms. In *Drosophila*, the central nervous system originates from the ventrolateral neurogenic region of the ectoderm (for reviews, see Campos-Ortega and Hartenstein, 1985; Campos-Ortega and Jan, 1991). In wild-type embryos, the neuroblasts, the progenitor cells of the embryonic nervous system, delaminate from the epithelial layer, move interiorly and divide to produce the neurons of the brain and the ventral nerve cord. Cells remaining in the epithelial layer of the neurogenic region adopt a different developmental fate. These cells develop as epidermoblasts, which give rise to ventral epidermis and secrete the embryonic cuticle.

This choice of cell fate in the neurogenic region is under genetic control (Artavanis-Tsakonas et al., 1991; Campos-Ortega and Jan, 1991; Campos-Ortega and Knust, 1990). In embryos homozygous for null mutations of the *Notch* gene, essentially all of the cells in the neurogenic region become neuroblasts (Lehman et al., 1983; Poulson, 1937). Such embryos die with a vast hypertrophy of the nervous system and a corresponding absence of epidermal structures. Embryos homozygous for null mutations of at least five other zygotically acting loci (*Delta*, *Enhancer of split*, *mastermind*, *big brain* and *neuralized*) exhibit a similar mutant phenotype (Lehman et al., 1983). Cell ablation studies during neurogenesis in grasshopper embryos, which have a mode of neural development similar to *Drosophila* embryos, have indicated that cell-cell interactions are important in the decision of a bipotential precursor cell to develop as either a neuroblast or an epidermoblast (Doe and Goodman, 1985).

Genetic and biochemical analyses of the *Notch* gene have suggested that *Notch* could play a role in the cell-cell interactions important in determining cell fate in the neurogenic region (Fehon et al., 1990, 1991; Kidd et al., 1989; Rebay et al., 1991; Xu et al., 1990; for reviews, see Artavanis-Tsakonas and Simpson, 1991; Artavanis-Tsakonas et al., 1991; Campos-Ortega and Jan, 1991; Greenspan, 1990; Greenwald and Rubin, 1992). The *Notch* gene encodes a large transmembrane protein whose extracellular domain contains 36 tandemly repeated copies of an epidermal growth factor (EGF)-like sequence (Kidd et al., 1986; Wharton et al., 1985). EGF-like sequences have been shown to act as sites for protein-protein interactions (Apella et al., 1987; Kurosawa et al., 1988), and these sequences are present in a wide variety of proteins (Bevilacqua et al., 1989; Jones et al.,

1988; Lawler and Hynes, 1986; Mann et al., 1989; Montell and Goodman, 1988; Siegelman et al., 1989; Sudhof et al., 1985; Suzuki et al., 1987; for a review, see Davis, 1990). The extracellular domain also contains three repeats of another cysteine-rich motif, termed the *Notch/lin-12* repeat. This motif is found in the *Notch* gene as well as in two genes in *Caenorhabditis elegans*, *lin-12* and *glp-1* (Yochem and Greenwald, 1989). These two genes, which encode transmembrane proteins which also contain EGF-like repeats in their extracellular domain, are involved in cell-cell interactions and cell fate specification during nematode development (for reviews, see Greenwald and Rubin, 1992; Maine and Kimble, 1990). The intracellular domains of *Notch*, as well as *lin-12* and *glp-1*, also contain six copies of another motif termed the *cdc10/ankyrin* repeats. These repeats have been identified on an increasingly broad range of proteins (Aves et al., 1985; Breeden and Nasmyth, 1987; Davis and Bennett, 1990; Lux et al., 1990; Ohno et al., 1990; Spence et al., 1990; Thompson et al., 1991), and have recently been shown to be domains involved in protein-protein interaction (Davis and Bennett, 1990; Thompson et al., 1991).

The isolation of murine homologs of genes important for development in other organisms has been an important addition to the array of techniques that can be used to study mammalian development (Chisaka and Capecchi, 1991; Joyner et al., 1991; McMahon and Bradley, 1990; for reviews, see Gridley, 1991; Kessel and Gruss, 1990). To determine if a gene homologous to *Drosophila Notch* plays a role in early mouse development, we have screened an 8.5 dpc mouse embryo cDNA library with probes from the *Xenopus Notch* homolog, *Xotch* (Coffman et al., 1990). We report here the isolation of a partial cDNA clone encoding the mouse homolog of *Notch*, which we have termed *Motch.* We characterize by ribonuclease protection analysis the expression pattern of *Motch* RNA in adult mice and postimplantation mouse embryos, and the induction of *Motch* RNA levels in tissue culture cells undergoing differentiation. An analysis by in situ hybridization of the spatial organization of *Motch* RNA expression suggests multiple roles for the *Motch* gene in patterning and differentiation in the early postimplantation mouse embryo.

Materials and methods

cDNA and genomic cloning

To isolate clones that cross-hybridize with the *Xotch* cDNA (Coffman et al., 1990), an 8.5 dpc mouse embryo cDNA library (Fahrner et al., 1987) and a mouse genomic library (Kinloch et al., 1988) were screened with two *Xotch* cDNA probes at low stringency. Plaque lifts were hybridized in 5 × SSC, 50 mM Tris-HCl, pH 8.0, 2.5 × Denhardt's, 100 μg ml^{-1} yeast RNA, 10% dextran sulphate, 1.0% SDS, 1.0% sodium pyrophosphate at 55ºC, and final wash conditions were 1 × SSC, 0.2% SDS at 55ºC. The probes were a 900 bp *Eco*RV-*Eco*RV fragment encoding amino acids 1111-1445 of the predicted Xotch protein (which includes part of the EGF-like repeat region) (Coffman et al., 1990), and a 500 bp *Eco*RV-*Bgl* II fragment encoding amino acids 1446-1612 (which includes the *Notch/lin-12* repeat region). One cDNA (c195) was isolated that hybridized with the *Notch/lin-12* repeat probe, and one genomic (g1) phage was isolated that hybridized with both *Xotch* probes. DNA fragments isolated from recombinant phage carrying cDNA or genomic sequences were subcloned into the plasmid vector pGem7 (Promega). DNA sequencing was performed by the dideoxy technique using the Sequenase enzyme (US Biochemical Corporation).

Ribonuclease protection analysis

Total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform technique (Chomczynski and Sacchi, 1987) using RNazol B (Biotecx). Ribonuclease protection analysis was performed with the RPA II Ribonuclease Protection Assay kit (Ambion), using ^{32}P-labelled antisense strand of the *Eco*R1-*Kpn*1 fragment of the *Motch* cDNA clone c195 as the antisense riboprobe. Quantitation of ribonuclease protection results was performed on a Betascope 603 blot analyzer (Betagen). The *Motch* signal (counts per minute) was normalized to the β-actin signal, and the highest normalized signal was arbitrarily set at 100 units. Results were similar if the *Motch* signal was normalized to a second housekeeping gene, the product of the *Mov-34* locus (Gridley et al., 1990, 1991). The results shown are representative of an experiment repeated four times.

Culture of P19 cells

P19 embryonal carcinoma cells were cultured and induced to differentiate essentially as described (Rudnicki and McBurney, 1987). Cells were grown in αMEM plus 10% fetal bovine serum. To induce differentiation, cells were placed in suspension culture in agarose-coated dishes, either as a single cell suspension or as lightly trypsinized aggregates of cells (as described for embryonic stem cells by Robertson, 1987). Cells placed in suspension culture were either in αMEM plus 10% fetal bovine serum (untreated controls) or in the same media plus 0.3 μM retinoic acid (Sigma). After four days in suspension, all samples contained aggregates of P19 cells. These aggregates were then plated out in 10 cm tissue culture dishes in media without retinoic acid. Microscopic examination of these cultures after five days revealed the presence of cells containing neuronal processes in retinoic acid-induced cultures, but not in untreated control cultures. RNA was harvested from these cells using RNAzol B, and RNA samples were assayed for *Motch* and β-actin transcripts by ribonuclease protection.

In situ hybridization

In situ hybridization was performed essentially as described by Wilkinson and Green (1990). Briefly, C57Bl/6 embryos were dissected and fixed at 4ºC in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight. The fixed embryos were dehydrated, embedded in paraffin, and 6 μm sections were cut and floated onto 3-aminopropyltriethoxysilane-coated slides. For hybridization, slides were dewaxed in xylenes, hydrated in an ethanol series and fixed in fresh 4% paraformaldehyde in PBS. Sections were treated with 20 μg ml^{-1} proteinase K in 50 mM Tris-HCl, 5 mM EDTA, pH 8.0, washed in PBS and postfixed in 4% paraformaldehyde in PBS. Sections were then treated with acetic anhydride, washed and dehydrated. [^{35}S]UTP-labelled single-stranded sense and antisense RNA probes were prepared by standard procedures (Sambrook et al.,1989). The probe was hydrolyzed to an average length of 100 bases, unincorporated nucleotides were removed by chromatography on a Nick column (Pharmacia), and the probe was ethanol-precipitated. The probe was resuspended at a concentration of 2 ng $μl^{-1}$ kb^{-1} in 100 mM DTT. The probe was then diluted 1:10 in hybridization solution (50% formamide, 0.3 M NaCl, 20 mM Tris-HCl, 5 mM EDTA, pH 8.0, 10% dextran sulphate, 1 × Denhardt's, 0.5 mg ml^{-1} yeast RNA), giving a final probe concentration of 0.2 ng $μl^{-1}$ kb^{-1}. After sections were hybridized

overnight at 55ºC, they were treated with ribonuclease A, washed at high stringency (50% formamide, 2 × SSC, 10 mM DTT at 65ºC) and dehydrated. Slides were dipped in NTB2 emulsion (Eastman Kodak) and exposed for approximately two weeks. Exposed slides were developed in D19 (Kodak), stained in 0.5% toluidine blue and mounted with Permount (Fisher). Double exposure (dark-field with red filter, bright-field with blue filter) photomicrographs were taken on a Zeiss Axioplan microscope.

Results

Isolation of a Motch cDNA clone

To determine if a gene homologous to *Drosophila Notch* plays a role in early mouse development, we screened mouse embryo cDNA (Fahrner et al., 1987) and genomic libraries at low stringency with cDNA probes encoding two domains of *Xotch*, the *Xenopus Notch* homolog (Coffman et al., 1990). One *Xotch* probe encoded part of the EGF-like repeat region of *Xotch*, while the other probe encoded the *Notch/lin-12* repeat region. This screen yielded a cDNA clone that hybridized to the *Xotch* probe encoding the *Notch/lin-12* repeat region. The 2.0 kb insert from this cDNA clone, c195, was subcloned and sequenced. Fig. 1A shows the nucleotide and translated amino acid sequences for the 291 bp *Eco*R1 - *Kpn*1 fragment at the 5′ end of this cDNA clone. After the initiation of these studies, the cloning of *Notch* homologs from human (Ellisen et al., 1991) and rat (Weinmaster et al., 1991) was reported. Comparison of the sequence of the mouse cDNA clone with the sequences of *Notch* and the other vertebrate *Notch* homologs confirms that this clone encodes the mouse *Notch* homolog, which we have named *Motch*. This *Eco*R1 - *Kpn*1 fragment encodes the last 13 amino acids of the third *Notch/lin-12* repeat, as well as the succeeding 84 amino acids of the *Motch* gene. Fig. 1B compares the translated amino acid sequence of this subclone with the amino acid sequences for *Notch* and the other published *Notch* homologs cloned from *Xenopus*, rat and man. The last line in Fig. 1B displays the consensus sequence for this region of the *Notch* family of proteins. In this region approximately 26% (26/98) of the amino acids are identical in all the *Notch* homologs, while 56% (55/98) of the amino acids are identical in all the vertebrate *Notch* homologs. Comparing the mouse and rat *Notch* homologs, 95% (92/97) of the amino acids in this region are identical. The *Eco*R1 - *Kpn*1 fragment of the *Motch* gene described above was subcloned and used to analyze *Motch* RNA expression by ribonuclease protection and in situ hybridization. Cloning of a full-length cDNA of the *Motch* transcript is in progress and will be described in a separate report. In addition, screening of a mouse genomic phage library (Kinloch et al., 1988) with the two *Xotch* probes also yielded a single clone which hybridized to both *Xotch* probes. Preliminary nucleotide sequence analysis of this genomic clone indicates that it contains part of the *Motch* gene encoding some of the EGF-like repeats as well as the *Notch/lin-12* repeats (data not shown).

Motch *expression in adult mouse tissues, embryos and in differentiating P19 cells*

Motch RNA expression in adult mouse tissues was analyzed utilizing a ribonuclease protection assay, using the cDNA fragment shown in Figure 1A as an antisense riboprobe. *Motch* transcripts were detected in a wide variety of adult tissues and included derivatives of all three germ layers (Fig. 2A). *Motch* RNA levels were highest in brain, lung and thymus. Lower levels of RNA were detected in spleen, bone marrow, spinal cord, eyes, mammary gland, liver, intestine, skeletal muscle (not shown) and heart. These data agree well with the expression pattern reported for 10-20 week old human fetuses analyzed for expression of the human *Notch* homolog, TAN-1 (Ellisen et al., 1991). *Motch* RNA expression during embryonic development was also examined with the ribonuclease protection assay. *Motch* RNA was present during all post-implantation stages examined (from 7.5 dpcto 16.5 dpc) (Fig. 2B).

We were interested in determining if *Motch* RNA levels were altered in cell culture systems that can undergo differentiation. Suspension culture of P19 embryonal carcinoma cells in the presence of retinoic acid leads to the differentiation of neuronal and glial cell types upon subsequent

A

```
AGTGCCGAATGTGAGTGGGATGGCCTAGACTGTGCTGAGCATGTACCCGAGCGGCTGGCA    60
SerAlaGluCysGluTrpAspGlyLeuAspCysAlaGluHisValProGluArgLeuAla

GCGGGCACCCTGGTCCTGGTGGTGCTGCTTCCACCCGACCAGCTACGGAACAACTCCTTC   120
AlaGlyThrLeuValLeuValValLeuLeuProProAspGlnLeuArgAsnAsnSerPhe

CACTTTCTGCGGGAGCTCAGCCACGTGCTGCACACCAACGTGGTCTTCAAGCGTGATGCG   180
HisPheLeuArgGluLeuSerHisValLeuHisThrAsnValValPheLysArgAspAla

CAAGGCCAGCAGATGATCTTCCCGTACTATGGCCACGAGGAAGAGCTGCGCAAGCACCCA   240
GlnGlyGlnGlnMetIlePheProTyrTyrGlyHisGluGluGluLeuArgLysHisPro

ATCAAGCGCTCTACAGTGGGTTGGGCCACCTCTTCACTGCTTCCTGGTACC            291
IleLysArgSerThrValGlyTrpAlaThrSerSerLeuLeuProGlyThr
```

B

```
M  SAECEWDGLDCAEHVPER-LAAGTLVLVVLLPP
R  SAECEWDGLDCAEHVPER-LAAGTLVLVVLLPP
N  SAECEWDGLDCAEHVPER-LAAGTLVVVVLMPP
X  NAECEWDGLDCANMPEN--LAEGTLVLVVLMPP
D  NAECSWDGLDCENKTQSPVLAEGAMSVVMLMNV
Con AECeWDGLDCa          LA  Gtl  vVvL  pp

M  DQLRNNSFHFLRELSHVLHTNVVFKRDAQGQQM
R  DQLRNNSFHFLRDVSHVLHTNVVFKRDAQGQQM
N  EQLRNSSFHFLRELSRVLHTNVVFKRDAHGQQM
X  ERLKNNSVNFLRELSRVLHTNVVFKKDSKGEYK
D  EAFREIQAQFLRNMSHMLRTTVRLKKDALGHDI
Con  l  n  s   FLR   S  vLhTnVvfK  D   G

M  IFPYYGHEEELRKHPIKRSTVGWATSSLLPGT
R  IFPYYGREEELRKHPIKRSAVGWATTSLLPGT
N  IFPYYGREEELRKHPIKRAAEGWAAPDALLGQ
X  IYPYYGNEEELKKHHIKRSTDYWSDAPSAIFS
D  IINWKDNVRV---PEIE-DTDF----------
Con I  pyyg  eeel  kh  Ikr      w
```

Fig. 1. Nucleotide and translated amino acid sequence of portion of *Motch* cDNA clone used for expression analysis. (A) Nucleotide and deduced amino acid sequence of the *Eco*R1-*Kpn*1 fragment of the *Motch* cDNA clone c195. Amino acids encoding the end of the third *Notch/lin-12* repeat are underlined. (B) Comparison of *Notch* gene family members. The amino acid sequence of the *Motch* cDNA subclone shown in A is compared to the *Notch* gene homologs isolated from rat (Weinmaster et al., 1991), human (Ellisen et al., 1991), *Xenopus* (Coffman et al., 1990) and *Drosophila Notch* (Kidd et al., 1986; Wharton et al., 1985). The last line displays a consensus sequence for this region of the *Notch* family of proteins. Amino acids in upper case are identical in all *Notch* homologs, while amino acids in lower case are identical in all vertebrate *Notch* homologs.

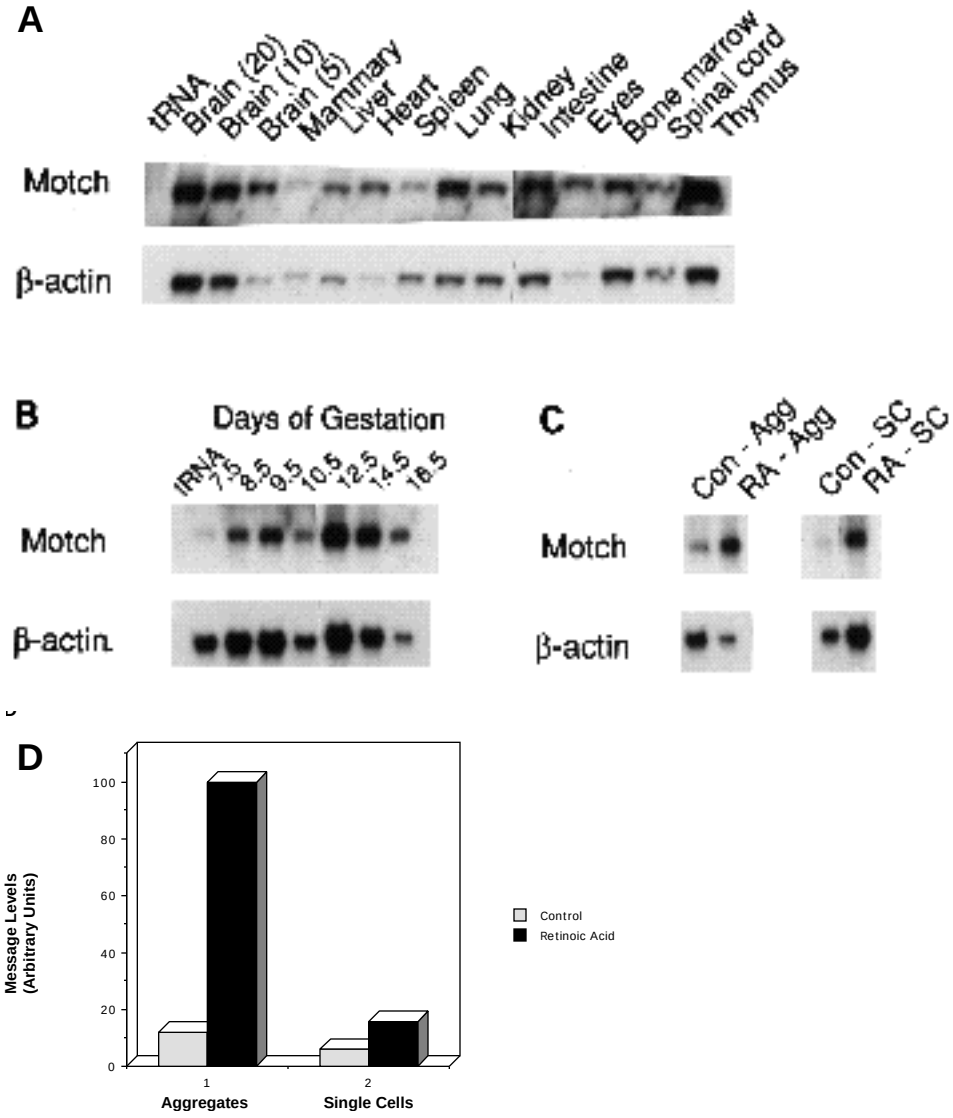

Fig. 2. *Motch* RNA expression. (A) Expression in adult mouse tissues. 10 μg of total RNA from the indicated tissues was analyzed in a ribonuclease protection assay using the *Motch* cDNA fragment shown in Fig. 1A as an antisense riboprobe. For brain RNA, the number of μg of total RNA analyzed is indicated. To control for RNA integrity and loading, samples were also analyzed with a β-actin riboprobe. (B) Expression in postimplantation mouse embryos. 10 μg of total RNA isolated from embryos at the indicated day of gestation was analyzed as described in A. (C) Induction of *Motch* transcript levels in differentiating P19 embryonal carcinoma cells. 10 μg of total RNA isolated from untreated control (Con) and retinoic acid-treated (RA) P19 cells was analyzed as above. The P19 cells were initially placed in suspension culture as single cells (SC) or as small aggregates of cells (Agg), as described in Materials and methods. (D) Quantitation of *Motch* RNA induction in differentiating P19 cells. Data from the experiment shown in C was quantitated using a Betascope 603 blot analyzer (Betagen). *Motch* RNA levels were determined as described in Materials and methods. The data shown here are a representative sample of an experiment repeated four times. *Motch* messenger RNA levels were similar if results were normalized to a second housekeeping gene, the product of the *Mov-34* locus (Gridley et.al., 1990, 1991).

plating of the cells (Rudnicki and McBurney, 1987). *Motch* RNA levels were determined by ribonuclease protection before and after induction of differentiation of P19 cells. The *Motch* gene is expressed in undifferentiated P19 cells. *Motch* RNA levels (normalized to RNA levels for β-actin - see Materials and methods) increased approximately 8-fold after induction with retinoic acid (Fig. 2C,D). The increase in *Motch* RNA levels was similar if the signal was normalized

Fig. 3. *Motch* RNA expression in embryos at 6.5 and 7.5 dpc. All sections were hybridized with a *Motch* antisense riboprobe. (A) Sagittal section of 6.5 dpc embryo. No *Motch* expression is observed in any embryonic or extra-embryonic region of the embryo. *Motch* is expressed at this time in maternally derived cells, primarily at the mesometrial pole of the decidua (not shown). (B) A slightly oblique sagittal section of 7.5 dpc embryo. *Motch* expression is observed in mesoderm and in embryonic ectoderm adjacent to the primitive streak. (C) Transverse section of 7.5 dpc embryo. Expression is again observed in mesoderm and embryonic ectoderm. *Motch* expression in the mesoderm appears to be graded. The highest levels are in the primitive streak, and they appear to decline in more lateral and anterior mesoderm (compare B). The open arrows delimit the anterior limits of extension of the mesodermal wings. Expression in the decidua is more widespread than at 6.5 dpc, and is now observed in maternally derived cells surrounding the embryo. am, amnion; e, embryonic ectoderm; ec, ectoplacental cone; ee, extra-embryonic ectoderm; m, mesoderm; ps, primitive streak.

Fig. 6. *Motch* expression in 13.5 dpc embryos. All sections were hybridized with a *Motch* antisense riboprobe. (A) Parasagittal section of the nasal region of an embryo at 13.5 dpc. Expression is observed in epidermis and developing whisker follicles. At this stage of gestation, *Motch* appears to be expressed in all surface ectoderm. (B) Higher magnification view of the section shown in A. *Motch* expression in the developing whisker follicle is confined to the inner root sheath. (C) Sagittal section through the eye of an embryo at 13.5 dpc. *Motch* expression is observed in the sensory layer of the retina and in the lens. Apparent expression in the pigment layer of the retina (asterisk) is an artifact due to light scattering by the pigment granules. (D) Higher magnification view of the section shown in C. *Motch* expression is not observed in differentiating cells of the ganglion cell layer of the retina. *Motch* expression is observed in mitotic cells in the sensory layer of the retina and in epithelial cells of the presumptive cornea. *, pigment layer of the retina; e, epidermis; gc, ganglion cell layer of the retina; ir, inner root sheath; l, lens; pc, presumptive cornea; r, retina; wf, whisker follicle.

Fig. 5. *Motch* RNA expression in embryos at 9.5 and 10.5 dpc. All sections were hybridized with a *Motch* antisense riboprobe. (A-C) Sections of a 9.5 dpc embryo. (D-H) Sections of a 10.5 dpc embryo. (A) Very high levels of expression are observed in presomitic mesoderm. Expression is also observed in the neural tube and in endothelial cells lining the dorsal aorta. (B) *Motch* is widely expressed in the developing brain, and also appears to be expressed in the meninges (arrow). (C) *Motch* is expressed in endocardium of the heart and in endothelial cells lining blood vessels in the branchial arches (arrows). (D) In this section *Motch* expression is observed in the spinal cord, in the condensing dorsal root ganglia, in mesonephric tubules, and in intersomitic blood vessels (arrows). (E) Higher magnification view of the section shown in (D). Expression is observed in endothelial cells lining the intersomitic blood vessels (arrows) and in the sclerotome. No expression is observed in dermatome or myotome. (F) *Motch* expression is observed in the mitotic cells of the ventricular zone of the spinal cord, and in the condensing dorsal root ganglia. No expression is observed in the floor plate or in postmitotic cells of the ventral horn of the spinal cord. (G) *Motch* is expressed in the trigeminal ganglion. (H) High levels of expression are observed in surface ectoderm of the lateral nasal process. Lower levels of expression are observed in ectoderm and mesenchyme of the medial nasal process. d, dermatome; da, dorsal aorta; dr, condensing dorsal root ganglion; e, endocardium; fp, floor plate; lnp, lateral nasal process; m, myotome; mes, mesencephalon; mnp, medial nasal process; ms, mesonephric tubules; op, olfactory pit; s, scleratome; tel, telencephalon; tg, trigeminal ganglion; vh, ventral horn of the spinal cord; vz, ventricular zone.

to a second housekeeping gene, the product of the *Mov-34* locus (Gridley et al., 1990, 1991). *Motch* RNA induction was consistently greater if the P19 cells were initially placed in suspension culture as aggregates, rather than as a single cell suspension.

Analysis of spatial localization of Motch *transcripts by in situ hybridization*

To further characterize *Motch* RNA expression during postimplantation development, we analyzed the spatial distribution of *Motch* transcripts by in situ hybridization. Since we were interested in determining if the *Motch* gene might play similar roles in early embryonic development of *Drosophila* and mice (e.g., cell fate determination in the developing nervous system), we concentrated our analysis on early postimplantation stages (6.5 dpc to 10.5 dpc). No *Motch* transcripts could be detected in any embryonic tissue at 6.5 dpc (Fig. 3A), although *Motch* transcripts were detected in maternally derived cells at the mesometrial pole of the decidua at this time (data not shown). At 7.5 dpc, *Motch* transcripts were first detected in mesoderm and in posterior embryonic ectoderm (Fig. 3B,C), in and adjacent to the primitive streak. No transcripts were detected in any extraembryonic tissues (visceral endoderm, amnion, chorion, ectoplacental cone) at this time.

At 8.5 dpc the highest level of *Motch* expression was in presomitic mesoderm (Fig. 4B-E). *Motch* expression was down-regulated when mesoderm condensed to form somites (Fig. 4F). *Motch* transcripts were also abundant in cephalic mesenchyme (Fig. 4D,E) and in endothelial cells lining the inner heart tube and the dorsal aorta (Fig. 4D,E). Much lower levels of *Motch* transcripts were present in neuroepithelium. Expression in neuroepithelium at 8.5 dpc appeared to be at about the limit of detection in our in situ hybridization experiments.

By 9.5 dpc, however, neuroepithelial tissues were a major site of *Motch* expression, and transcripts were detected in many areas of the brain and neural tube (Fig. 5A,B). *Motch* RNA levels remained high in presomitic mesoderm (Fig. 5A). We continued to detect *Motch* transcripts in endothelial cells at 9.5 and 10.5 dpc; transcripts could be observed in endocardium (Fig. 5C) and in endothelial cells lining the dorsal aorta (Fig. 5A), the intersomitic blood vessels (Fig. 5D,E) and the aortic branches in the branchial arches (Fig. 5C). At 10.5 dpc we continued to detect *Motch* transcripts in neuroepithelium. *Motch* expression in the spinal cord was confined to mitotic cells of the ventricular zone; postmitotic cells located in the ventral horns did not express (Fig. 5D,F). We also detected transcripts in mesonephric tubules (Fig. 5D), condensing dorsal root ganglia (Fig. 5D,F), the trigeminal ganglion (Fig. 5G) and the lateral nasal process (Fig. 5H). In a less extensive analysis of embryos at 13.5 dpc, we detected expression in surface ectoderm, in the eye and in the developing whisker follicles (Fig. 6). Expression in the whisker follicle was confined to mitotic cells of the inner root sheath (Fig. 6B). Similarly, *Motch* expression in the eye was confined to mitotic cells of the lens, corneal epithelium and sensory layer of the retina. Differentiating ganglion cells of the neural retina did not express *Motch* (Fig. 6D).

Discussion

We report here an analysis of the RNA expression pattern of *Motch*, a mouse homolog of *Drosophila Notch.* After the initiation of these studies, the cloning of *Notch* homologs from human (Ellisen et al., 1991) and rat (Weinmaster et al., 1991) was reported. The vertebrate *Notch* gene family also includes *Xotch*, the *Xenopus Notch* homolog (Coffman et al., 1990) and a second mouse gene, the product of the *int-3* locus (Gallahan et al., 1987), which is more distantly related to *Notch* than the genes mentioned above (Robbins et al., 1992). The vertebrate *Notch* genes are very well conserved and appear to be true homologs of *Notch* : all the apparent structural motifs of *Notch* (the EGF-like repeats, the *Notch/lin-12* repeats and the *cdc10/ankyrin* repeats) are conserved, both in the number of repeats present and the location and order of these structural motifs in the protein. Even a skeleton of the *opa* repeat, the polyglutamine stretch present in a number of *Drosophila* genes, is retained. Thus, it seems quite likely that the biochemical mode of action will be similar in all the *Notch* homologs. Given the strong structural conservation of the *Notch* family of genes, we were interested in determining if the mode of action of these proteins was functionally conserved as well. In particular, we wanted to determine if *Motch*, the mouse *Notch* homolog, was involved in early differentiative events of the developing mouse embryo, e.g. formation of the mesoderm or the neural plate. As a first step to answering this question, we examined by in situ hybridization the RNA expression pattern of the *Motch* gene during early postimplantation development (6.5 -10.5 dpc).

RNA expression pattern of Motch

Our results showed that, in postimplantation embryos, *Motch* transcripts were first detected at 7.5 dpc in mesoderm and posterior embryonic ectoderm, in and adjacent to the primitive streak. This region of the embryonic ectoderm consists of epithelial cells destined to delaminate and form mesoderm and definitive endoderm (Beddington, 1981; Beddington, 1982; Tam and Beddington, 1987). *Motch* transcripts at 8.5 dpc were most abundant in presomitic mesoderm, cephalic mesenchyme and endothelial cells of the endocardium and dorsal aorta, but were barely detectable in neuroepithelium itself at this stage. A day later, however, neuroepithelial tissues have become one of the major sites of *Motch* expression in the embryo. Thus, *Motch* expression may not be required in the cells of the neural plate for their specification and initial differentiation. However, *Motch* is expressed in neuroepithelium after it first forms.

In addition to neuroepithelium of the central nervous system, we also observed *Motch* expression in a number of other sites. Several of these sites are derived from neural crest cells. *Motch* expression was observed in the condensing dorsal root ganglion at 10.5 dpc. Other sites of *Motch* expression, such as the trigeminal ganglion and the cephalic mesenchyme, are also derived at least in part from neural crest cells. As mentioned previously, another site of *Motch* expression was endothelium, which is of mesodermal origin.

Our results support and extend the expression data published for the other vertebrate *Notch* homologs. Coffman et al. (1990) found, using a ribonuclease protection assay, that *Xotch*, the *Xenopus* homolog, was expressed almost uniformly in early embryos and was present in all three prospective germ layers. Weinmaster et al. (1991) used the rat *Notch* homolog to examine expression in mouse embryos from 9.5 to 16.5 dpc by in situ hybridization. They observed strong expression in developing brain, spinal cord, eyes, dorsal root ganglia and trigeminal ganglia between 10 and 12 dpc. Expression in later embryos was observed in tissues undergoing epithelial-mesenchymal interactions, such as whisker follicles, tooth buds and kidney (Weinmaster et al., 1991). We also observed expression in eyes and whisker follicles at 13.5 dpc.

We further observed that *Motch* transcript levels increase when P19 embryonal carcinoma cells undergo differentiation into neuronal cell types. Since *Motch* RNA is expressed at low levels in undifferentiated P19 cells, it is not clear at present if this induction of *Motch* RNA levels has any functional significance. We plan further experiments to modulate *Motch* expression in P19 cells to determine if this has an effect on the ability of these cells to differentiate.

Proposed models of Motch *function*

Our expression results suggest that *Motch* is playing multiple roles during development of mouse embryos and in the adult. This is consistent with the involvement of *Notch* in several developmental events in *Drosophila* besides neuroblast segregation (Cagan and Ready, 1989; Hartenstein and Posakony, 1990; Portin, 1975; Ruohola et al., 1991). Our results further suggest that the first site of action of the *Motch* gene during mouse embryogenesis is in mesoderm. This result is interesting in light of recent findings on the functioning of the neurogenic genes in mesoderm of the *Drosophila* embryo. Corbin et al. (1991) demonstrated that in addition to specifying cell fate in the neurogenic ectoderm, *Notch* and the other neurogenic genes also appear to be required for correct specification of cell fates in mesoderm of *Drosophila* embryos.

While it seems quite likely that the biochemical mode of action will be similar in all the *Notch* homologs, it is not entirely clear at present what role *Notch* actually plays in *Drosophila* development. Several models have been proposed for *Notch* function. One proposed role for *Notch* is that it functions as the receptor of the signal for lateral inhibition. *Notch* has been shown to function autonomously in both embryonic (Hoppe and Greenspan, 1990) and imaginal (de Celis et al., 1991; Heitzler and Simpson, 1991; Markopoulou and Artavanis-Tsakonas, 1991) development, as would be expected for a receptor. Autonomy has also been demonstrated for the *Notch*-related genes in *C. elegans*, *lin-12* and *glp-1* (Austin and Kimble, 1987; Seydoux and Greenwald, 1989). Most, but not all (Technau and Campos-Ortega, 1987; however, see Simpson, 1990), of the experimental results obtained to date support the model that *Notch* functions autonomously, probably as the receptor for a signal. This would not exclude, however, the possibility that *Notch* may have additional functions as well. The early expression pattern of *Motch* does not seem to support a role in binary cell fate decisions, although a mutational analysis of the *Motch* gene will be required to help answer this question.

Another function that has been proposed for *Notch* is a role in cell proliferation. Both in situ hybridization and immunohistochemistry experiments have demonstrated a good, although not absolute, correlation during *Drosophila* development between *Notch* expression and mitotically active cell populations (Kidd et al., 1989; Markopoulou and Artavanis-Tsakonas, 1989). Both our results and those of Coffman et al. (1990) and Weinmaster et al. (1991) indicate that the vertebrate *Notch* genes also show a correlation, particularly in the developing nervous system, between expression and cell proliferation. In addition, while the *Xenopus*, rat and mouse *Notch* homologs were all cloned by low-stringency hybridization techniques, the human *Notch* homolog, TAN-1, and the product of the mouse *int-3* locus were identified as oncogenes. TAN-1 was identifed at a translocation breakpoint in a T cell lymphoma (Ellisen et al., 1991), while the *int-3* locus is a common integration site for Mouse Mammary Tumor Virus (MMTV)-induced mammary tumors (Gallahan et al., 1987; Robbins et al., 1992). In both cases it appears that deregulated expression of the cytoplasmic domain, containing the *cdc10/ankyrin* repeats, is the likely cause of the tumorigenic phenotype. This has been particularly well established for the *int-3* locus, since a minigene construct containing essentially only the cytoplasmic domain under the transcriptional control of the MMTV long terminal repeat can cause mammary tumors in transgenic mice (Jhappan et al., 1992). It is clear, then, that mutations of vertebrate *Notch* genes can affect cell proliferation. Whether the normal function of these genes involves a role in cell proliferation remains to be demonstrated.

Another model that has been proposed for *Notch* is that its expression is required to stabilize and/or maintain the differ-

entiated state (Hoppe and Greenspan, 1990). It is possible that *Motch* expression may also be serving this function. For example, *Motch* would not be required for specification and initial formation of neuroepithelium, but would be required for its maintenance, hence the widespread expression of *Motch* in neuroepithelium at 9.5 dpc.

A fourth function that has been proposed for *Notch* is a role in cell adhesion (Cagan and Ready, 1989; Greenspan, 1990; Hoppe and Greenspan, 1990). The RNA expression pattern of *Motch*, however, gives no indication that it plays a role, at least initially, in cell adhesion. In a number of instances, *Motch* RNA is expressed in mesenchymal or migrating cells (e.g. invaginating mesoderm and cephalic mesenchyme). It will be important to analyze expression of *Motch* protein to determine if it is expressed concomitantly with the first appearance of *Motch* transcripts in mesodermal cells.

The expression pattern of the *Motch* gene suggests that it plays an important role in patterning and differentiation of early postimplantation mouse embryos. Proof of such a role, however, will require a functional analysis in transgenic mice. Analysis of both gain-of-function (i.e. ectopic expression) and loss-of-function (i.e. construction of null mutations in embryonic stem cells) mutations will greatly aid our understanding of *Motch* gene function in early mouse development.

We would like to thank Jill McMahon, Galya Vassileva, and Mary Dickinson for advice on in situ hybridization, Clark Coffman, Chris Kintner and Bill Harris for the *Xotch* probes, Ross Kinloch and Paul Wassarman for the mouse genomic phage library, and Joe Grippo for use of his microscope. F.F. del A. was supported by a fellowship from the Ministerio de Educación y Ciencia of Spain (#FP90-32450447).

References

Apella, E., Robinson, E. A., Ullrich, S. J., Stoppelli, M. P., Corti, A., Cassani, G. and Blasi, F. (1987). The receptor-binding sequence of urokinase. *J. Biol. Chem.* **262**, 4437-4440.

Artavanis-Tsakonas. S., Delidakis, C. and Fehon, R. G. (1991). The *Notch* locus and the cell biology of neuroblast segregation. *Ann. Rev. Cell Biol.* **7**, 427-452.

Artavanis-Tsakonas, S. and Simpson, P. (1991). Choosing a cell fate: a view from the *Notch* locus. *Trends Genet.* **7**, 403-408.

Austin, J. and Kimble, J. (1987). *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans*. *Cell* **51**, 589-599.

Aves, S. J., Durkacz, B. W., Carr, A. and Nurse, P. (1985). Cloning, sequencing, and transcriptional control of the *Schizosaccharomyces pombe cdc10* start gene. *EMBO J.* **4**, 457-463.

Beddington, R. S. P. (1981). An autoradiographic analysis of the potency of embryonic ectoderm in the 8th day postimplantation mouse embryo. *J. Embryol. exp. Morph.* **64**, 87-104.

Beddington, R. S. P. (1982). An autoradiographic analysis of tissue potency in different regions of the embryonic ectoderm during gastrulation in the mouse. *J. Embryol. exp. Morph.* **69**, 265-285.

Bevilacqua, M. P., Stengelin, S., Gimbrone, M. A. and Seed, B. (1989). Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* **243**, 1160-1165.

Breeden, L. and Nasmyth, K. (1987). Similarity between cell-cycle genes of budding yeast and fission yeast and the *Notch* gene of *Drosophila*. *Nature* **329**, 651-654.

Cagan, R. L. and Ready, D. F. (1989). *Notch* is required for successive cell decisions in the developing *Drosophila* retina. *Genes Dev.* **3**, 1099-1112.

Campos-Ortega, J. A. and Hartenstein, V. (1985). *The Embryonic Development of* Drosophila melanogaster. Berlin: Springer Verlag.

Campos-Ortega, J. A. and Jan, Y. N. (1991). Genetic and molecular bases of neurogenesis in *Drosophila melanogaster*.*Ann. Rev. Neurosci.* **14**, 399-420.

Campos-Ortega, J. A. and Knust, E. (1990). Genetics of early neurogenesis in *Drosophila melanogaster*. *Ann. Rev. Genet.* **24**, 387-407.

Chisaka, O. and Capecchi, M. R. (1991). Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene *hox-1.5*. *Nature* **350**, 473-479.

Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156-159.

Coffman, C., Harris, W. and Kintner, C. (1990). *Xotch*, the *Xenopus* homolog of *Drosophila Notch*. *Science* **249**, 1438-1441.

Corbin, V., Michelson, A. M., Abmayr, S. M., Neel, V., Alcamo, E., Maniatis, T. and Young, M. W. (1991). A role for the *Drosophila* neurogenic genes in mesoderm differentiation. *Cell* **67**, 311-323.

Davis, C. G. (1990). The many faces of epidermal growth factor repeats. *New Biologist* **2**, 410-419.

Davis, L. H. and Bennett, V. (1990). Mapping the binding sites of human erythrocyte ankyrin for the anion exchanger and spectrin. *J. Biol. Chem.* **265**, 10589-10596.

de Celis, J. F., Mari-Beffa, M. and Garcia-Bellido, A. (1991). Cell-autonomous role of *Notch*, an epidermal growth factor homologue, in sensory organ differentiation in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **88**, 632-636.

Doe, C. Q. and Goodman, C. S. (1985). Early events in insect neurogenesis. II. The role of cell interactions and cell lineage in the determination of neuronal precursor cells. *Dev. Biol.* **111**, 206-219.

Ellisen, L. W., Bird, J., West, D. C., Soreng, A. L., Reynolds, T. C., Smith, S. D., and Sklar, J. (1991). TAN-1, the human homolog of the *Drosophila Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**, 649-661.

Fahrner, K., Hogan, B. L. M. and Flavell, R. A. (1987). Transcription of the H-2 and Qa genes in embryonic and adult mice. *EMBO J.* **6**, 1265-1271.

Fehon, R. G., Johansen, K., Rebay, I. and Artavanis-Tsakonas, S. (1991). Complex cellular and subcellular regulation of *Notch* expression during embryonic and imaginal development of *Drosophila*: implications for *Notch* function. *J. Cell Biol.* **113**, 657-669.

Fehon, R. G., Kooh, P. J., Rebay, I., Regan, C. L., Xu, T., Muskavitch, M. A. T. and Artavanis-Tsakonas, S. (1990). Molecular interactions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in *Drosophila*. *Cell* **61**, 523-534.

Gallahan, D., Kozak, C. and Callahan, R. (1987). A new common integration region (*int-3*) for mouse mammary tumor virus on mouse chromosome 17. *J. Virol.* **61**, 218-220.

Greenspan, R. J. (1990). The *Notch* gene, adhesion, and developmental fate in the *Drosophila* embryo. *New Biologist* **2**, 595-600.

Greenwald, I. and Rubin, G. M. (1992). Making a difference: the role of cell-cell interactions in establishing separate identities for equivalent cells. *Cell* **68**, 271-281.

Gridley, T. (1991). Insertional versus targeted mutagenesis in mice. *New Biologist* **3**, 1025-1034.

Gridley, T., Gray, D. A., Orr-Weaver, T., Soriano, P., Barton, D. E., Francke, U. and Jaenisch, R. (1990). Molecular analysis of the *Mov-34* mutation: transcript disrupted by proviral integration in mice is conserved in *Drosophila*. *Development* **109**, 235-242.

Gridley, T., Jaenisch, R. and Gendron-Maguire, M. (1991). The murine *Mov-34* gene: full-length cDNA and genomic organization. *Genomics* **11**, 501-507.

Hartenstein, V. and Posakony, J. W. (1990). A dual function of the *Notch* gene in *Drosophila* sensillum development. *Dev. Biol.* **142**, 13-30.

Heitzler, P. and Simpson, P. (1991). The choice of cell fate in the epidermis of *Drosophila*. *Cell* **64**, 1083-1092.

Hoppe, P. E. and Greenspan, R. J. (1990). The *Notch* locus of *Drosophila* is required in epidermal cells for epidermal development. *Development* **109**, 875-885.

Jhappan, C., Gallahan, D., Stahle, C., Chu, E., Smith, G. H., Merlino,

G. and Callahan, R. (1992). Expression of an activated *Notch*-related *Int-3* transgene interferes with cell differentiation and induces neoplastic transformation in mammary and salivary glands. *Genes Dev.* **6**, 345-355.

Jones, F. S., Burgoon, M. P., Hoffman, S., Crossin, K. L., Cunningham, B. A. and Edelman, G. M. (1988). A cDNA clone for cytotactin contains sequences similar to epidermal growth factor-like repeats and segments of fibronectin and fibrinogen. *Proc. Natl. Acad. Sci. USA* **85**, 2186-2190.

Joyner, A. L., Herrup, K., Auerbach, B. A., Davis, C. A. and Rossant, J. (1991). Subtle cerebellar phenotype in mice homozygous for a targeted deletion of the *En-2* homeobox. *Science* **251**, 1239-1243.

Kessel, M. and Gruss, P. (1990). Murine developmental control genes. *Science* **249**, 374-379.

Kidd, S., Baylies, M. K., Gasic, G. P. and Young, M. W. (1989). Structure and distribution of the Notch protein in developing Drosophila. *Genes Dev.* **3**, 1113-1129.

Kidd, S., Kelley, M. R. and Young, M. W. (1986). Sequence of the *Notch* locus of *Drosophila melanogaster*: relationship of the encoded protein to mammalian clotting and growth factors. *Mol. Cell. Biol.* **6**, 3094-3108.

Kinloch, R. A., Roller, R. J., Fimiani, C. M. and Wassarman, P. A. (1988). Primary structure of the mouse sperm receptor polypeptide determined by genomic cloning. *Proc. Natl. Acad. Sci. USA* **85**, 6409-6413.

Kurosawa, S., Stearns, D. J., Jackson, K. W. and Esmon, C. T. (1988). A 19-kDa cyanogen bromide freagment from the epidermal growth factor homology domain of rabbit thrombomodulin contains the primary thrombin binding site. *J. Biol. Chem.* **263**, 5993-5996.

Lawler, J. and Hynes, R. O. (1986). The structure of human thrombospondin, an adhesive glycoprotein with multiple calcium-binding sites and homologies with several different proteins. *J. Cell Biol.* **103**, 1635-1648.

Lehman, R., Jimenez, F., Dietrich, U. and Campos-Ortega, J. A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster. Wilhelm Roux's Arch. Dev. Biol.* **192**, 62-74.

Lux, S. E., John, K. M. and Bennett, V. (1990). Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. *Nature* **344**, 36-42.

Maine, E. M. and Kimble, J. (1990). Genetic control of cell communication in *C. elegans* development. *BioEssays* **12**, 265-271.

Mann, K., Deutzmann, R., Aumailley, M., Timpl, R., Raimondi, L., Yamada, Y., Pan, T., Conway, D. and Chu, M. (1989). Amino acid sequence of mouse nidogen, a multidomain basement membrane protein with binding activity for laminin, collagen IV and cells. *EMBO J.* **8**, 65-72.

Markopoulou, K. and Artavanis-Tsakonas, S. (1989). The expression of the neurogenic locus *Notch* during the postembryonic development of *Drosophila melanogaster* and its relationship to mitotic activity. *J. Neurogenetics* **6**, 11-26.

Markopoulou, K. and Artavanis-Tsakonas, S. (1991). Developmental analysis of the *facets*, a group of intronic mutations at the *Notch* locus of *Drosophila melanogaster* that affect postembryonic development. *J. Exp. Zool.* **257**, 314-329.

McMahon, A. P. and Bradley, A. (1990). The *wnt-1* (*int-1*) proto-oncogene is required for development of a large region of the mouse brain. *Cell* **62**, 1073-1085.

Montell, D. J. and Goodman, C. S. (1988). *Drosophila* substrate adhesion molecule: sequence of laminin B1 chain reveals domains of homology with mouse. *Cell* **56,** 463-473.

Ohno, H., Takimoto, G. and McKeithan, T. W. (1990). The candidate proto-oncogene *bcl-3* is related to genes implicated in cell lineage determination and cell cycle control. *Cell* **60**, 991-997.

Portin, P. (1975). Allelic negative complementation of the *Abruptex* locus of *Drosophila melanogaster*. *Genetics* **81**, 121-123.

Poulson, D. F. (1937). Chromosomal deficiencies and embryonic development of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **23**, 133-137.

Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, L., Cherbas, P. and Artavanis-Tsakonas, S. (1991). Specific EGF repeats of *Notch* mediate interactions with *Delta* and *Serrate*: implications for *Notch* as a multifunctional receptor. *Cell* **67**, 687-699.

Robbins, J., Blondel, B. J., Gallahan, D. and Callahan, R. (1992). Mouse mammary tumor gene *Int-3*: a member of the *Notch* gene family transforms mammary epithelial cells. *J. Virol.* **66**, 2594-2599.

Robertson, E. J. (1987). Embryo-derived stem cell lines. In *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach* (ed. E. J. Robertson), pp. 71-112. Oxford: IRL Press.

Rudnicki, M. A. and McBurney, M. W. (1987). Cell culture methods and induction of differentiation of embryonal carcinoma cell lines. In *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach* (ed. E. J. Robertson), pp. 19-49. Oxford: IRL Press.

Ruohola, H., Bremer, K. A., Baker, D., Swedlow, J. R., Jan, L. Y. and Jan, Y. N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell* **66**, 433-449.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press.

Seydoux, G. and Greenwald, I. (1989). Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans*. *Cell* **57**, 1237-1245.

Siegelman, M. H., van de Rijn, M. and Weissman, I. L. (1989). Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. *Science* **243**, 1165-1172.

Simpson, P. (1990). *Notch* and the choice of cell fate in *Drosophila* neuroepithelium. *Trends Genet.* **6**, 343-345.

Spence, A. M., Coulson, A. and Hodkin, J. (1990). The product of *fem-1*, a nematode sex-determining gene, contains a motif found in cell cycle control proteins and receptors for cell-cell interactions. *Cell* **60**, 981-990.

Sudhof, T. C., Goldstein, J. L., Brown, M. S. and Russell, D. W. (1985). The LDL receptor gene: a mosaic of exons shared with different proteins. *Science* **228**, 815-822.

Suzuki, K., Kusumoto, H., Deyashiki, Y., Nishioka, J., Maruyama, I., Zushi, M., Kawahara, S., Honda, G. T., Yamamoto, S. and Horiguchi, S. (1987). Structure and expression of human thrombomodulin receptor on endothelium acting as a cofactor for protein C activation. *EMBO J.* **6**, 1891-1897.

Tam, P. P. L. and Beddington, R. S. P. (1987). The formation of mesodermal tissues in the mouse embryo during gastrulation and early organogenesis. *Development* **99**, 109-126.

Technau, G. M. and Campos-Ortega, J. A. (1987). Cell autonomy of expression of neurogenic genes of *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA* **84**, 4500-4504.

Thompson, C. C., Brown, T. A. and McKnight, S. L. (1991). Convergence of *Ets*- and *Notch*-related structural motifs in a heteromeric DNA binding complex. *Science* **253**, 762-768.

Weinmaster, G., Roberts, V. J. and Lemke, G. (1991). A homolog of *Drosophila Notch* expressed during mammalian development. *Development* **113**, 199-205.

Wharton, K. A., Johansen, K. M., Xu, T. and Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* **43**, 567-581.

Wilkinson, D. G. and Green, J. (1990). In situ hybridization and the three-dimensional reconstruction of serial sections. In *Postimplantation Mammalian Embryos: A Practical Approach* (eds. A. J. Copp and D. L. Cockroft), pp. 155-171. Oxford: IRL Press.

Xu, T., Rebay, I., Fleming, R. J., Scottgale, T. N. and Artavanis-Tsakonas, S. (1990). The *Notch* locus and the genetic circuitry involved in early *Drosophila* neurogenesis. *Genes Dev.* **4**, 464-475.

Yochem, J. and Greenwald, I. (1989). *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in *C. elegans*, encode similar transmembrane proteins. *Cell* **58**, 553-563.

(Accepted 7 April 1992)

Note aded in proof:

The nucleotide sequence of the Motch cDNA clone c195 will appear in the EMBL/Genbank/DBJ databases under the accession number Z11886.

DEV5853 colour caption

Fig. 4. *Motch* expression in 8.5 *dpc* embryos. All sections were hybridized with a *Motch* antisense riboprobe. (A) Schematic diagram of a mouse embryo at 8.5 dpc. The approximate levels of sections B-G are indicated. The extent of the foregut diverticulum is indicated by the dotted line. (B-F) Transverse sections. Highest levels of *Motch* expression are observed in presomitic mesoderm (B-E). *Motch* expression is substantially down-regulated when the paraxial mesoderm condenses to form somites (F). *Motch* transcripts are also abundant in mesenchyme (B-E) and endocardium (D,E). (G) Slightly oblique frontal section. *Motch* expression is observed in cephalic mesenchyme, but little or no expression is observed in neuroepithelium. cm, cephalic mesenchyme; cs, condensed somite; e, endocardial cells forming inner tube of the primitive heart; f, foregut; pm, presomitic mesoderm.