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Differential expression of mammalian Numb, Numblike and Notch1 suggests distinct roles during mouse cortical neurogenesis

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SUMMARY

During *Drosophila* neurogenesis, asymmetric cell divisions are achieved by differential segregation of Numb (d-Numb) into one of the daughter cells to cause a bias in the *Notch* mediated cell-cell interaction. We have isolated a second mammalian gene with significant sequence similarity to *d-numb*, mouse *numblike*. When expressed in dividing neural precursors in *Drosophila*, Numblike is symmetrically distributed in the cytoplasm, unlike endogenous d-Numb or expressed mouse Numb (m-Numb), both of which are asymmetrically localized to one half of the cell membrane. In *d-numb* loss-of-function mutant embryos, expression of Numblike allows both daughter cells of a neural precursor to adopt the fate of the cell that normally inherits d-Numb. In mice, *numblike* mRNA is preferentially expressed in adult and embryonic nervous system. In the developing neocortex, Numblike is expressed in postmitotic neurons in the cortical plate, but not in progenitors within the ventricular zone where m-Numb and Notch1 are expressed. We have also found that, in dividing cortical progenitors, Notch1 is distributed around the entire membrane, unlike m-Numb which is asymmetrically localized to the apical membrane. We propose that an interplay between cell-intrinsic mechanisms (executed by *m-numb* and *numblike*) and cell-extrinsic mechanisms (mediated by *Notch1*) may be involved in both progenitor cell proliferation and neuronal differentiation during mammalian cortical neurogenesis.

Key words: mouse *numblike*, *numb*, *Notch*, asymmetric cell division, neurogenesis

INTRODUCTION

During mammalian neurogenesis, neural progenitors and postmitotic neurons occupy distinct regions of the neurogenic epithelium which form the walls of the embryonic neural tube. Neural progenitors occupy the inner layer (ventricular zone), whereas newborn neurons migrate outwards, away from the luminal surface, and settle in the outer layer for terminal differentiation. Such cellular events have been well documented in the developing neocortex and appear to be conserved in other regions of the central nervous system (CNS) (reviewed by Rakic, 1988; McConnell, 1995). Although various genes have been shown to be specifically expressed either in ventricular precursors or in differentiating neurons, little is known about the molecular mechanism that regulates whether ventricular daughter cells remain as progenitors or opt for terminal differentiation.

In the developing neocortex, cortical progenitors undergo mitosis with their nuclei at the ventricular surface. Retroviral lineage tracing experiments in rodents and primates have shown that a given cortical progenitor can generate neurons over multiple divisions at different times of development, suggesting that cortical neurogenesis is, at least partly, achieved through asymmetric divisions, in which one daughter cell of a cortical progenitor becomes a postmitotic neuron while the other remains as a precursor cell (Luskin et al., 1988; Price and Thurlow, 1988; Walsh and Cepko, 1988; Kornack and Rakic, 1995; Reid et al., 1995; reviewed by Rakic, 1988; Caviness et al., 1995; McConnell, 1995). Imaging of DiI-labeled ventricular cells in living ferret brain slices using time-lapse confocal microscopy has provided more direct evidence that cortical progenitors may indeed divide asymmetrically and that cell cleavage planes may predict whether a cortical progenitor divides symmetrically or asymmetrically (Chenn and McConnell, 1995). When a cortical progenitor divides with the cell cleavage plane roughly parallel to the ventricular surface, it generates two daughter cells that differ in their overall morphology and in the speed with which their nuclei move away from the luminal surface. The basal daughter cell loses its luminal contact and its nucleus moves rapidly towards the cortical plate, at a rate similar to that of the migration of young postmitotic neurons. The apical daughter cell, however, retains its luminal contact and its nucleus moves more slowly. It has been proposed that the basal daughter cell differentiates into a postmitotic neuron in the cortical plate while its apical counterpart remains as a progenitor cell within the ventricular zone (Chenn and McConnell, 1995). Recent findings indicate that some evolutionarily conserved molecular mechanisms may be responsible for specifying distinct daughter cell fates in such asymmetric divisions (Zhong et al., 1996).

During *Drosophila* neurogenesis, *d-numb* and *Notch* are two of the genes involved in distinct daughter cell fate specification during asymmetric divisions of neural precursors. *d-numb* encodes a membrane-associated protein that is asymmetrically localized to one pole of dividing neural precursors so as to be predominantly segregated to only one of the daughter cells after division. Differential segregation of d-Numb is required for the two daughter cells to adopt distinct fates (Uemura et al., 1989; Rhyu et al., 1994; Spana et al., 1995). Notch is a transmembrane receptor that mediates cell-cell interactions in various developmental pathways (reviewed by Artavanis-Tsakonas et al., 1995). Antagonistic roles of *Notch* and *d-numb* in asymmetric divisions of neural precursors are suggested by the finding that *Notch* mutant phenotypes are opposite to *d-numb* mutant phenotypes (Hartenstein and Posakony, 1990; Guo et al., 1996; Spana and Doe, 1996; Frise et al., 1996). Furthermore, d-Numb can interact physically with the Notch intracellular domain (Guo et al., 1996). It has therefore been suggested that d-Numb functions, at least partly, by causing a bias in the Notch mediated cell-cell interaction, possibly via direct physical interaction with Notch (Jan and Jan, 1995; Guo et al., 1996). Thus it appears that asymmetric cell divisions are achieved through interactions between cell-intrinsic and cell-extrinsic mechanisms.

We have recently isolated a highly conserved mammalian homologue of *d-numb*, *m-numb* (Zhong et al., 1996). During mouse cortical neurogenesis, m-Numb is asymmetrically localized to the apical membrane of dividing ventricular cells. Therefore, in an asymmetric horizontal division, m-Numb is segregated predominantly to the apical daughter cell that remains as a progenitor. Furthermore, like its *Drosophila* counterpart, m-Numb can physically interact with the intracellular domain of the mammalian Notch homologue, Notch1, which is also present in cortical progenitors. We have postulated that mechanisms for asymmetric divisions during neurogenesis are evolutionarily conserved and that the presence of m-Numb in apical daughter cells suppresses Notch activity in these cells, thereby allowing them to remain as progenitors. Presumably, the higher *Notch1* activity in the basal daughter cell allows it to take on a fate different from that of a neural progenitor. Once it has migrated to the cortical plate, the basal daughter cell may require other factors to regulate *Notch* signaling before it can terminally differentiate into a neuron, since *Notch* signaling usually directs cells towards nonneuronal fates (reviewed by Artavanis-Tsakonas et al., 1995).

Here we report the cloning and characterization of mouse *numblike (nbl)*, which has extensive sequence similarity to *m-numb* and *d-numb*. We show that, when expressed in dividing neural precursors in *Drosophila*, Nbl is symmetrically distributed in the cytoplasm. We further show that, while it fails to enable neural precursors to divide asymmetrically, *nbl* allows both their daughter cells to adopt the fate determined by d-Numb. In mice, *nbl* and *m-numb* have distinct but overlapping expression patterns both in the adult and during embryogenesis. We show that in the developing neocortex, Nbl is expressed in terminally differentiating neurons in the cortical plate, but not in progenitor cells within the ventricular zone, where both m-Numb and Notch1 are expressed. We also show that, in dividing cortical progenitors, while m-Numb is asymmetrically localized to the apical membrane, Notch1 is distributed around the entire cell membrane. These results may have important implications for mechanisms of progenitor cell proliferation and neuronal differentiation during mammalian neurogenesis.

MATERIALS AND METHODS

Isolation of a second mouse *numb* homologue

A 2.6 kb rat cDNA, which encodes an open reading frame of 607 amino acids, including a stretch of 290 amino acids that shares 63.7% identity with d-Numb (Zhong et al., 1996), was used to screen a mouse midgestation cDNA library (a gift from G. Martin, UCSF) under moderate stringency [hybridization at 65°C overnight in a buffer (Church and Gilbert, 1984) containing approx. 10^6 cpm/ml of ^{32}P-labeled probe with the highest stringency wash in 2× SSC/0.1% SDS at 60°C for 1 hour]. A 2.6 kb mouse *nbl* cDNA was isolated. The deduced amino acid sequence shares 98% identity with that of the rat cDNA, suggesting that they are homologues.

Antiserum production and immunofluorescence

For antiserum production, a peptide corresponding to amino acids 570-603 of Nbl was synthesized. A cysteine residue was added at the N terminus for coupling to carrier keyhole limpet hemocyanin. Antibodies were raised in rabbits using standard procedures by Caltag Corporation, South San Francisco, California. The resultant antiserum (anti-MNBL1) was affinity purified using its immunogen coupled to Sulfolink columns (Pierce). However, while the antiserum strongly recognizes Nbl, it also cross-reacts with m-Numb due to the high level of conservation between these two proteins in this region. On immunoblots using adult mouse tissue extracts and in vitro translated proteins, anti-MNBL1 specifically recognized both Nbl and m-Numb, but no other proteins (W. Zhong, unpublished observation). Therefore, Nbl protein expression patterns are recognized by comparing the staining pattern of anti-MNBL1 to the staining pattern of anti-MNBR1, which specifically recognizes m-Numb (Zhong et al., 1996), and *nbl* mRNA expression patterns. The anti-Notch1 antibody is a rabbit antiserum against the intracellular domain of Notch1 and affinity purified.

Immunofluorescence staining of mouse sections or *Drosophila* embryos was as described by Zhong et al. (1996). Affinity-purified anti-MNBL1, anti-Notch1 and anti-Kv1.4 were diluted 1:1000 and immunoreactivities were visualized using biotinylated goat anti-rabbit IgG secondary antibodies and streptavidin-coupled fluorescein. Images were recorded using a Bio-Rad confocal microscope and merged using Adobe Photoshop.

Transgenic fly generation and analysis

The 2.6 kb rat *nbl* cDNA, which starts at amino acid 5, was fused in-frame with the *Xenopus β-globin* 5′UTR and MYC epitope (MEEKLISEED). The resultant DNA was cloned into pUAST and injected into embryos to generate transgenic flies (Brand and Perrimon, 1993).

For analysis of *nbl* expression, *UAS-nbl/Cyo* flies were crossed to *hairy-GAL4/TM3* flies (Brand and Perrimon, 1993) and embryos collected. For rescue experiments, *UAS-nbl/TM3*, *UAS-d-Numb/TM3* (provided by S. Wang) or *hairy-GAL4/TM3* flies were crossed to *numb^1/Cyo* flies (Uemura et al., 1989). The resultant *numb^1/+; UAS-nbl/+* or *numb^1/+; UAS-d-Numb/+* flies were crossed to *numb^1/+; hairy-GAL4/+* flies and embryos collected. Rescued embryos (*numb^1/numb^1; hairy-GAL4/UAS-nbl)* were recognized by double labeling with anti-MNBL1 and mAb22C10 or anti-Prospero.

RNA analysis and in situ hybridization

RNA preparation, northern blot analysis and T2 RNase protection assay were as described by Zhong et al. (1994). For the northern blot, a ^{32}P-labeled rat *nbl* fragment of 1.2 kb was used to detect *nbl* mRNA. The same blot was also probed with *GAPDH* as a control. For the T2 RNase protection assay, a ^{32}P-labeled antisense RNA probe of 0.5 kb (corresponding to the exon encoding amino acids 37-133 and the flanking intron sequences) was used.

Mouse embryos were fixed in 4% paraformaldehyde by overnight

immersion and sectioned (10-15 μm, cryostat sections for digoxigenin [DIG] probes and paraffin sections for ^{33}P probes). For in situ hybridization, antisense RNA probes corresponding to mouse *nbl* or *m-numb* (approx. 1.1 to 1.6 kb for DIG-labeled probes and approx. 0.5 kb for ^{33}P-labeled probes) were synthesized to detect *nbl* or *m-numb* transcripts on mouse tissues, using methods described elsewhere with minor modifications (Schaeren-Wiemers and Gerfin-Moser, 1993; Duncan et al., 1994).

Fusion protein production and protein binding assays

The production of GST fusion protein of the intracellular domain (ICD) of Notch1, in vitro translation of Numblike (full length MYC-rat-Numblike fusion used in transgenic fly production), protein binding assays and the yeast two-hybrid assay were as described (Zhong et al., 1996).

RESULTS

The mouse *numblike* gene

We isolated mouse *nbl* from a midgestation embryonic cDNA library (see Materials and Methods). This 2.6 kb cDNA encodes a protein of 603 amino acids (Fig. 1). The N-terminal portion of the protein (amino acids 42 to 331) shows strong sequence similarity to both m-Numb and d-Numb, with 76% and 63.7% identity, respectively (Fig. 2A). Like m-Numb and d-Numb, this region of the protein contains all key residues predictive of a phosphotyrosine binding (PTB) domain (Fig. 1 underlined; Zhou et al., 1995; Zhong et al., 1996). The C-terminal portion of Nbl also shows significant sequence similarity to m-Numb (46.7% identity, Fig. 2A). This segment of Nbl contains a stretch of 15 glutamine residues (Fig. 1), which are also present in its rat counterpart (23 residues) but not in m-Numb or d-Numb. Expansion of poly-glutamine repeats in various proteins has been implicated in the pathogenesis of several neurodegenerative diseases (reviewed by Ross, 1995). Overall, Nbl is more similar to m-Numb than d-Numb. The similarity between Nbl and m-Numb is present throughout the protein while the homology between Nbl and d-Numb is primarily confined to the N-terminal portion of the protein (Fig. 2B).

Nbl is a cytoplasmic protein unable to localize asymmetrically in *Drosophila* neural precursors

Unlike m-Numb and d-Numb, which are membrane associated proteins and can be asymmetrically localized in dividing neural precursors of *Drosophila* so as to be segregated primarily to one of the daughter cells (Rhyu et al., 1994; Zhong et al.,

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gcagcactcgggaagccgggcccaaccggggccgcgggaggcgggggcgcccgggccctggatgtcccgcagcgcggcggccagcggtgg 90
                                                                M  S  R  S  A  A  A  S  G  G  10
acccaggagacctgatcagcatttgtccccagccccctgtggggcctcgggggcccctgaaaccttcaggacggagtcagacggggcagg 180
 P  R  R  P  D  Q  H  L  S  P  A  P  C  G  A  S  G  A  P  E  T  F  R  T  E  S  D  G  A  G  40
caccatgaacaagttacggcagagtctgcggcggaggaagccagcctatgtgcctgaggcatcacgcccacaccagtggcaggcagatga 270
 T  M  N  K  L  R  Q  S  L  R  R  R  K  P  A  Y  V  P  E  A  S  R  P  H  Q  W  Q  A  D  E  70
ggatgcggtgcgcaaaggcacctgcagcttccctgttaggtacctgggccacgtggaagtggaggagtcccgggggaatgcacgtttgtga 360
 D  A  V  R  K  G  T  C  S  F  P  V  R  Y  L  G  H  V  E  V  E  E  S  R  G  M  H  V  C  E  100
agatgctgtgaagaagctgaaggcgatgggccggaagtccgtgaagtctgtcctgtgggtgtcagctgatgggctccgagtggtggatga 450
 D  A  V  K  K  L  K  A  M  G  R  K  S  V  K  S  V  L  W  V  S  A  D  G  L  R  V  V  D  D  130
caagaccaaggacctgctggtagaccagaccattgagaaggtgtccttctgtgctcctgatcgcaacctggacaaggctttctcctacat 540
 K  T  K  D  L  L  V  D  Q  T  I  E  K  V  S  F  C  A  P  D  R  N  L  D  K  A  F  S  Y  I  160
atgccgcgacggcaccacacgccgctggatctgccactgcttcctggccctcaaggactcgggtgagaggctgagccatgctgtgggctg 630
 C  R  D  G  T  T  R  R  W  I  C  H  C  F  L  A  L  K  D  S  G  E  R  L  S  H  A  V  G  C  190
cgcgttcgccgcctgcctggagagaaagcagcgacgggagaaggaatgcggagtcaccgccgccttcgacgccagtcgcaccagctttgc 720
 A  F  A  A  C  L  E  R  K  Q  R  R  E  K  E  C  G  V  T  A  A  F  D  A  S  R  T  S  F  A  220
ccgcgagggctccttccgcctgtcgggggcgggcggcctgcggagcgtgaggcggggacaagaagaaagaagcagcagctgctcctgc 810
 R  E  G  S  F  R  L  S  G  G  G  R  P  A  E  R  E  A  G  D  K  K  K  E  A  A  A  A  P  A  250
tgtggctcctggacctgcccagcctgggcacgtttctccgacaccagccaccacatccctggagagaagggagaggcgggcaccccagt 900
 V  A  P  G  P  A  Q  P  G  H  V  S  P  T  P  A  T  T  S  P  G  E  K  G  E  A  G  T  P  V  280
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 L  S  Q  K  N  S  P  F  K  R  Q  L  S  L  R  L  N  E  L  P  S  T  L  Q  R  R  T  D  F  Q  340
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 V  K  G  T  V  P  E  M  E  P  P  G  T  G  D  S  D  G  I  N  A  L  C  T  Q  I  S  S  S  F  370
cgccagcgctggggccccagcgtcagggccaccacctgccacaacagggacttctgcctggggtgagccctccgtacccgctgcagctgc 1260
 A  S  A  G  A  P  A  S  G  P  P  P  A  T  T  G  T  S  A  W  G  E  P  S  V  P  A  A  A  A  400
cttccagcctgggcacaagcggacaccctctgaggctgagcgatggttggaggaagtgtcccaggtggctaaggcccagcagcaacagca 1350
 F  Q  P  G  H  K  R  T  P  S  E  A  E  R  W  L  E  E  V  S  Q  V  A  K  A  Q  Q  Q  Q  Q  430
acagcagcagcagcaacaacagcagcagcaagccacctccgtgccaccaatgcccaccatggccccccaccctttcaaccctttctgcccc 1440
 Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  A  T  S  V  P  P  M  P  T  M  A  P  T  L  Q  P  F  S  A  P  460
agtggggccctttgacactgcagctgcccaagtggctgttttcctgccgcccacacatatgcagcctccgtttgtgcccgcctacccagg 1530
 V  G  P  F  D  T  A  A  A  Q  V  A  V  F  L  P  P  T  H  M  Q  P  P  F  V  P  A  Y  P  G  490
tctgggttatccaccaatgccccgggtgcctgtggtgggcatcacaccttcacagatggtagccaacgccttctgctcagctgcccagct 1620
 L  G  Y  P  P  M  P  R  V  P  V  V  G  I  T  P  S  Q  M  V  A  N  A  F  C  S  A  A  Q  L  520
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ccccaatggggctccctggcccccagagccagcgcctgcccctgcccctgagttggacccccttcgaggcccagtgggcggcactagaagg 1800
 P  N  G  A  P  W  P  P  E  P  A  P  A  P  A  P  E  L  D  P  F  E  A  Q  W  A  A  L  E  G  580
caaacccgctgtggagaaaccctccaacccctttctctggtgacttgcagaagaccttcgagattgaactgtagcccaaattgccctgctc 1890
 K  P  A  V  E  K  P  S  N  P  F  S  G  D  L  Q  K  T  F  E  I  E  L                       603
ccgctccatcagccccaggtgtaccgcgctcaggaggggaggcccagcctctatcctagtgttgctccctgagctgcgcccctcaatgct 1980
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ttcaagctactttccctagtttctgttgtgcccttccacctggcagtgttgggcagggtggaggagggaggcccacttaagcctggtccg 2250
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tgttgactctgttacctcatgtcggtgcccgctggggaggggtcccaggagaagaagaagggggtgcctggcgggtacttttctatcttt 2520
tatttccagatttttttgtatctaaacttgcagatttgtattatacaaggacagccaataaaggaagaatataatggtg            2600
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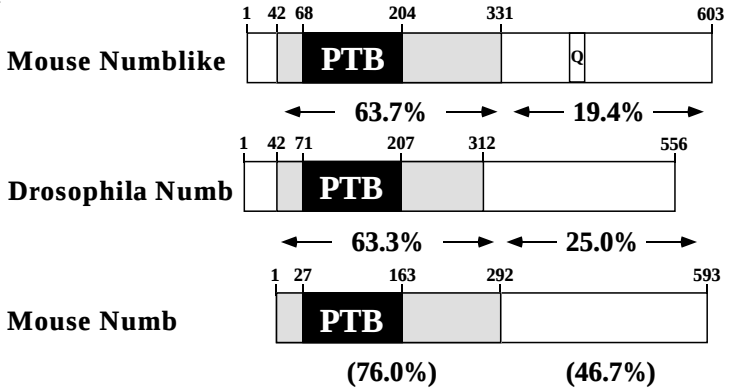
Fig. 1. Nucleotide sequences and the deduced amino acid sequence of mouse *numblike*. The first in-frame methionine is numbered 1. Underlined amino acids represent a presumptive PTB domain. Underlined nucleotides represent a potential polyadenylation signal. GenBank accession number for mouse *numblike* is U96441.

A



B

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    d-Numb  MGNSSSHTHE PLERGFTRGK FGDVKNGKSA SFRFSKKSPK KMDRLRRSFR 50
    m-Numb  .......... .......... .......... .......... .-NK--Q-=- 9
m-Numblike  +SR+AAASGG +RRPDQHLSP APCGAS+APE T++TESDGAG T-**--*-L- 50

    d-Numb  DSFRRRKDRV PESSKPHQWQ ADEEAVRSAT CSFSVKYLGC VEVFESRGMQ 100
    m-Numb  ...-KKDVY- --A-R----- T--=G--TGK ---P-=---H ---D-----H 56
m-Numblike  ...-+*PA*- --*-*----- +--D+--K*+ ---*-R---* ---E-----* 97

    d-Numb  VCEEALKVLR QSRRRPVRGL LHVSGDGLRV VDDETKGLIV DQTIEKVSFC 150
    m-Numb  I--D-V-R-K ATGKKA-KAV -W--A----- --EK--D-=- ---------- 106
m-Numblike  +--*-*-K-* *M*+*S-*S* -*--*----- --+*--*-L- ---------- 147

    d-Numb  APDRNHERGF SYICRDGTTR RWMCHGFLAC KDSGERLSHA VGCAFAVCLE 200
    m-Numb  -----FD=A- ---------- --I--C-M-V --T------- ------A--- 156
m-Numblike  -----L*K*- ---------- --*--*-+-L --+------- ------*--- 197

    d-Numb  RKQRRDKECG VTMTFDTKNS TFTRTGSFRQ QTLTERLAMA TVGTNERSVD 250
    m-Numb  ---K-E---- --A=--ASRT =-=-E----V T=A==QAERE EIMKQLQDAK 206
m-Numblike  ---+-*---- --*A--**** S-A-*----L SGGGRP**** AGD*KKEA*. 246

    d-Numb  .......... ...GPGSAMP GPPAATVKPF NP........ ........FA 271
    m-Numb  KAETDKTVVG PSVA--NTA- S=SSP-SPTP DGTASSEMNN P.......H- 249
m-Numblike  ........AA *A**--P+Q- +HV**-PA*T S+GEKG*AGT *VAAGTTAA- 288

    d-Numb  IERPHATPNM LERQSSFRLS TIGSQ..SPF KRQMSLRIND LPSNADRQRA 319
    m-Numb  -P-R--PIEQ -A--G---GF PAL--KM--- ---L---=-E ---TMQ-KTD 299
m-Numblike  -*-*--*L** -V--*---** ***--*N--- ---*---L-* ---*L*-R** 338

    d-Numb  FLAAAADNPL QTPLRSVSP. ....IAEVSP AKSAGADPSS AAAVAADSV. 363
    m-Numb  -PIKNTVPEV E....GEAES ISSLCSQITS =F=TPSEDPF SS=PMTKP=T 345
m-Numblike  -QV*G****M *P+GT*D+DG *NA**T**+* S*ASAGA+A+ GPP*+*TG.. 386

    d-Numb  .....SQLCQ ELSQGLSLLT QTDALLATGE DLNFNNN... .RSINQNIIA 404
    m-Numb  LVAPQ=PVL= GTEW-Q=SGA ASPG=FQA-H RRTPSEADRW LEEVSKSVR- 395
m-Numblike  .......... TSA*-EP*VP *AA+.**P-* K******E** *****+VAK- 425

    d-Numb  AEKQVQHVHS YAPPTAQVTP RVASTTPTYQ TL........ .......... 436
    m-Numb  QQP-=SAAPL QPVLQPPPPA AI=PPA-PF- GH.....AFL TSQPVPVGVV 440
m-Numblike  **Q-Q+QQQQ *QQQ*+TSV+ PMPTM*-+L- PFSAPVGP*D *AAAQVAVFL 475

    d-Numb  ...HSQSPSR SEQSIETSTE LP........ ....NAEQWL GHVVRSTSPA 471
    m-Numb  PP..L-PAFV PT==YPVANG M=YPA.SNVP VVGITPS-MV AN=FGTAGHP 487
m-Numblike  **T+M-*+** *..A**...* +G**PMPR** *******-** **A*C+*AQL 520

    d-Numb  APKRPTYLAN VGRAQTLAS. GTGAAVGGGG PDDPF..... .......... 505
    m-Numb  QTTH-HQSPS LAKQ=TFPQY ETSS-TTSPF FKP-AQHLNG SAAFNGVDNG 537
m-Numblike  *...-QPATL *+*+GA**PP AAP*-P++QA RPR-...... .......... 551

    d-Numb  ...DAEWVAN VAAAKQLSPD LPIPSTARSP LARHSTNPFI SPPKAP.... 548
    m-Numb  GLASGNRHAE =PPGT..C-. VDPFEAQWAA -ESK=KQRTN PS-TN-FSSD 584
m-Numblike  ..NG+P+PP* P+*+P..A-E +********* -*G*P...AV EK-S*-**G* 594

    d-Numb  .AQSFQVQL                                             556
    m-Numb  LQKT-EIE-                                             593
m-Numblike  ****-***-                                             603
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Fig. 2. Sequence comparison between *Drosophila* Numb and its mammalian Numb homologues. (A) Schematic diagrams of mouse Nbl, d-Numb and m-Numb. Percentages represent amino acid identity between adjacent proteins and those in parentheses between mouse Nbl and m-Numb. PTB is a presumptive phosphotyrosine binding domain and Q represents poly-glutamine repeats. (B) Sequence comparison between d-Numb, m-Numb and m-Numblike. Sequences were aligned using the GCG pileup program. (-) represents identical amino acids in all three proteins; (=) represents amino acids that are conserved between d-Numb and m-Numb; (*) represents amino acids conserved between m-Numb and Nbl; (+) represents amino acids conserved between d-Numb and Nbl; and (.) represents gap in the sequence.

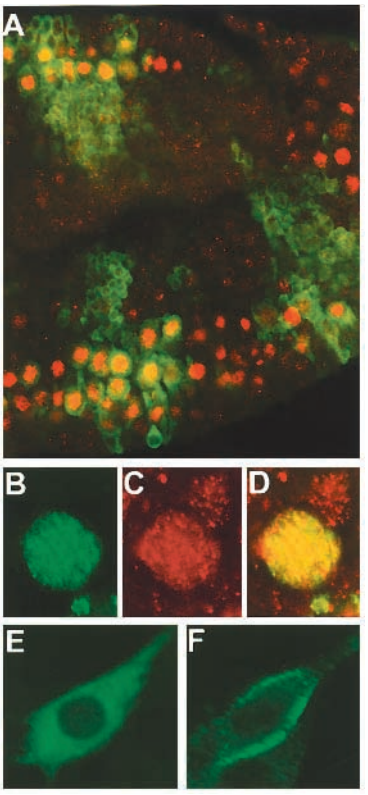

Fig. 3. Mouse Numblike is a cytoplasmic protein. (A-D) Subcellular localization of mouse Nbl (revealed by immunofluorescence, in green) in *Drosophila*. Red is Asense (Ase) staining used to identify neuroblasts or SOP cells. (A) stage 8; Nbl is expressed in alternate parasegments (see Materials and Methods). In a dividing CNS neuroblast (B-D), Nbl staining (B) is cytoplasmic, similar to Ase (C), a nuclear protein which becomes cytoplasmic due to the breakdown of the nuclear membrane during mitosis. (D) Superimposed images of B and C. (E-F) In mouse C2C12 cells, the exogenously introduced Nbl is cytoplasmic (E, in green) while m-Numb is membrane associated (F). Notice the clear space separating m-Numb staining and the nuclear shadow in F, which is absent in E.

1996), Nbl was not localized asymmetrically in neural precursors in *Drosophila*. As shown in Fig. 3, the expressed Nbl was symmetrically distributed throughout the cytoplasm in non-dividing neuroblasts of the CNS (Fig. 3A) and remained so even when these neural precursors underwent mitosis (Fig. 3B-D).

To test whether Nbl is also a cytoplasmic protein in mice, we performed immunofluorescence assays on cultured murine C2C12 myoblasts that had been infected with retroviral vectors expressing either *nbl* or *m-numb* (W. Zhong, unpublished data). Indeed, the expressed Nbl was distributed in the cytoplasm while m-Numb was localized to the plasma membrane (Fig. 3E and F, respectively).

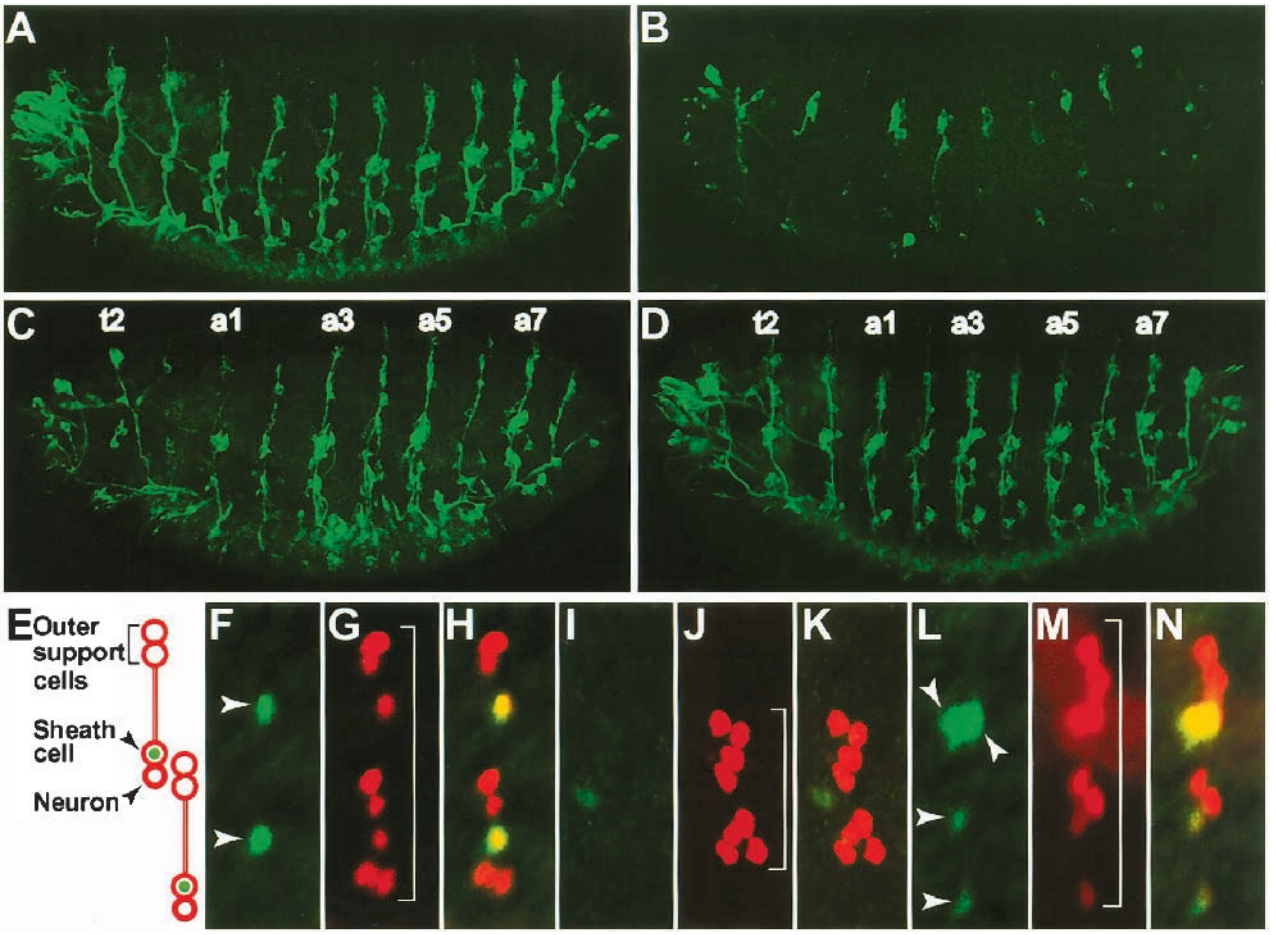

Fig. 4. Effects of mouse Numblike expression in *Drosophila numb* mutant embryos. Nbl is expressed in alternate parasegments (see Materials and Methods). (A-D) Neuronal rescue. PNS neurons visualized by mAb22C10 (in green) in wild-type (A), *numb* mutant (B), and *numb* mutant expressing either Nbl (C) or d-Numb (D). Embryos are arranged with anterior to the left and dorsal on top. (E-N) Sheath cell rescue. Two dorsal-most es organs (E, schematic) in abdominal hemisegments of embryos from wild-type (F-H), *numb* mutant (I-K) and *numb* mutant expressing Nbl (L-N). Green is Prospero staining identifying sheath cells (F,I,L, arrowheads), while red is Cut staining identifying all eight cells of the two es organs (G,J,M, bracketed). (H,K,N) Superimposed images of respective previous images in the series.

Ectopic *nbl* expression mimics *d-numb* misexpression phenotypes in *Drosophila* embryos

In contrast to m-Numb and d-Numb, the symmetric cytoplasmic localization of Nbl in dividing neural precursors in *Drosophila* results in the partition of Nbl to both daughter cells of a neural precursor. We then asked whether in each daughter cell Nbl could substitute for d-Numb in cell fate specification. Loss of *numb* function in *Drosophila* embryos results in a severe loss of neurons in the peripheral nervous system (PNS), which can be visualized by staining with the marker mAb22C10 (Fig. 4B; Uemura et al., 1989; Rhyu et al., 1994). This severe neuronal loss is caused by a change in the fate adopted by one of the sensory organ precursor (SOP) daughter cells, the IIb cell, which normally inherits d-Numb and produces a neuron and a sheath. In the absence of d-Numb, the IIb cell adopts the fate of its sister cell (IIa, see below). We expressed Nbl in these mutant embryos using a method which strongly promoted its expression in alternate parasegments (Brand and Perrimon, 1993; see Materials and Methods). As a result, the corresponding segments (t2, a1, a3, a5 and a7) no longer exhibited the neuronal loss characteristic of the *Drosophila numb* mutant (Fig. 4C).

Whereas the effects of Nbl expression superficially resembled those due to expression of d-Numb in the same segmental pattern (Fig. 4D), differences between Nbl and d-Numb actions became apparent upon further analysis of the formation of the two dorsal-most external sensory (es) organs in abdominal hemisegments, where the effect of d-Numb had been well characterized (Uemura et al., 1989; Rhyu et al., 1994). As schematized in Fig. 4E, each wild-type es organ consists of four cells, a neuron, a sheath cell and two outer support cells. During PNS neurogenesis, these four cells are the descendants of a single SOP cell through two rounds of asymmetric divisions. The SOP first divides into two cells, named ‘IIa’ and ‘IIb’. The IIa cell then divides to form the two outer support cells while the IIb cell gives rise to the neuron and the sheath cell. d-Numb is asymmetrically localized to only one half of the SOP cell membrane before division and segregated primarily to the IIb cell. In the absence of d-Numb, the IIb cell is transformed into a IIa cell, generating a mutant es organ with four outer support cells (Uemura et al., 1989; Rhyu et al., 1994). We used the anti-Cut antibody as a marker to recognize all eight cells of the two es organs (Fig. 4G, bracketed) and the anti-Prospero antibody to reveal the two sheath cells within this eight-cell cluster (Fig. 4F, arrows). In

numb mutant embryos, the sheath cells (as well as the neurons, not shown) were transformed into outer support cells, resulting in the loss of Prospero staining (Fig. 4I) without altering the number of Cut-positive cells (Fig. 4J, bracketed). In segments of mutant embryos where Nbl was expressed, however, there were four Prospero-positive cells (Fig. 4L, arrows), in contrast to the presence of two such cells in wild-type embryos or in segments of embryos that express m-Numb or d-Numb in the same segmental pattern (Zhong et al., 1996; data not shown). The number of Cut-positive cells remained at eight (Fig. 4M). This phenotype of Nbl expression is similar to that of d-Numb misexpression, driven by a heatshock promoter, in both daughter cells (Rhyu et al., 1994). To summarize, whereas asymmetric localization of d-Numb and m-Numb results in their segregation into one of the two daughter cells of the SOP so that it adopts the fate of the IIb cell, symmetric localization of Nbl causes both daughters of the SOP to inherit Nbl and become IIb cells (Rhyu et al., 1994; Zhong et al., 1996). This indicates that the IIb cell fate can be specified by introducing d-Numb, m-Numb or Nbl into that cell.

Differential expression of *nbl* and *m-numb* during mouse embryogenesis

nbl is expressed both during mouse embryogenesis and in the adult (Fig. 5A and B, respectively). The functional differences between *nbl* and *m-numb* when assayed in *Drosophila* neural precursor cells (Fig. 4) suggest that *nbl* may play roles somewhat different from *m-numb* during mammalian neurogenesis. To gain insight into such *nbl* functions, we further characterized *nbl* expression during mouse embryogenesis by performing in situ hybridization and immunohistochemistry on embryo sections (Fig. 6). While little *nbl* mRNA could be detected at E8.5 (E0.5 is noon of the day when the vaginal plugs are observed), it was clearly present in trigeminal ganglia and dorsal root ganglia by E9.5 (data not shown). At E12.5, *nbl* was highly expressed in the developing nervous system, with little or no expression elsewhere in the embryo (Fig. 6A-E). This is very different from *m-numb*, which is expressed at low levels in most embryonic structures, including the developing nervous system (Fig. 6F-H; Zhong et al., 1996). *nbl* mRNA was detected throughout the neural tube, from rostral telencephalon to caudal spinal cord. It was also expressed in all of the cranial sensory ganglia (V to XI), the dorsal root ganglia (DRG), the sympathetic ganglia, and the developing sensory organs such as nasal epithelia and retina (Fig. 6A-C, E and data not shown).

One important feature of *nbl* expression within the developing neural tube is the presence of *nbl* mRNA only in regions outside, but not within, the mitotically active ventricular zone. In the developing midbrain (Fig. 6D), for example, *nbl* mRNA was only present in the mantle zone, which contains postmitotic neurons that are undergoing active differentiation. In developing spinal cord, neurogenesis does not proceed uniformly, with a rostral (older) to caudal (younger) neurogenetic gradient. As a result, the rostral E12.5 spinal cord, which was near the end of neurogenesis, had a very thin ventricular zone and appeared to have a more diffuse pattern of *nbl* expression (Fig. 6E, top). In contrast, in the caudal spinal cord, where active neurogenesis was still underway, *nbl* mRNA (Figure 6C and E, bottom) and protein (Fig. 6K) were limited to the mantle zone, including the differentiating motor neurons of the ventral horn. m-Numb, however, was also very abundant

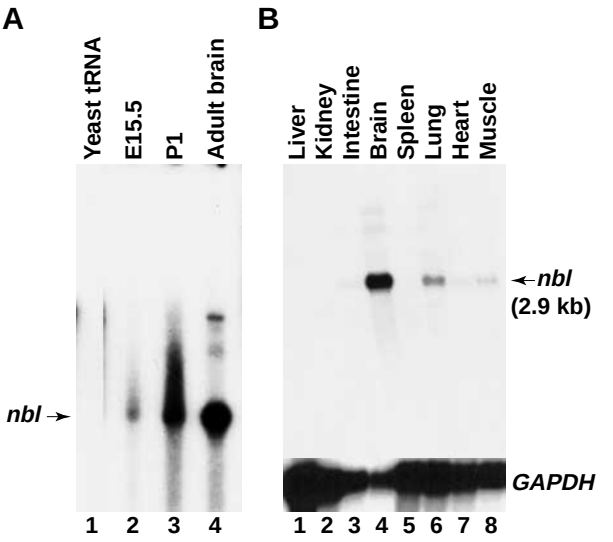

Fig. 5. *numblike* expression in adult and embryonic mice. (A) T2 RNase protection assay of *nbl* expression throughout mouse development. 20 μg of total cytoplasmic RNA from whole embryos is used from each stage. Yeast tRNA is used as a control. High molecular mass bands in lane 4 represent partially spliced *nbl* RNA. (B) Northern blot analysis of *nbl* expression in adult mouse organs. 5 μg of poly(A)$^+$ RNA is used in each lane. A *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* probe is used as a control. High molecular mass bands in lane 4 represent partially spliced *nbl* RNA.

in the germinal ventricular zone (Fig. 6L). Such differential expression of *nbl* and *m-numb* could also be observed in other regions of the developing neural tube and sensory organs (nasal epithelia and retina; Figs 6, 7 and data not shown).

Distribution of Nbl, m-Numb and Notch1 proteins in developing mouse neocortex

The potential interaction between *numb* and *Notch* functions during neurogenesis in *Drosophila* and mice prompted us to further investigate the distribution of Nbl, m-Numb and Notch1 during cortical neurogenesis. In the developing mouse neocortex, Nbl immunoreactivity was mainly present in the cortical plate (Fig. 7A), which contains only postmitotic neurons that are undergoing active differentiation. The low level of staining in the ventricular zone is likely due to cross-reaction to m-Numb, since the part of the Nbl protein used for immunogen is well conserved between m-Numb and Nbl and the antiserum recognizes both proteins on immunoblots (see Materials and Methods and Fig. 2). This is consistent with the observation that *nbl* mRNA is only detected in cells at the cortical plate (Fig. 7A, insert). In contrast, Notch1 immunoreactivity was higher in cells of the ventricular zone, with less protein present at the cortical plate (Fig. 7G), consistent with the distribution of *Notch1* mRNA observed in both mice and rats (Weinmaster et al., 1991, 1992; Lardelli et al., 1994). However, m-Numb was expressed in all layers of the cortical neuroepithelium, from the ventricular zone to the cortical plate (Fig. 7B; Zhong et al., 1996).

In interphase cells within the ventricular zone, both Notch1 and m-Numb staining appeared to be symmetrically distributed. As expected, the Notch1 immunoreactivity appeared to be membrane-bound. Since the extra-nuclear space is very thin in non-dividing cortical progenitors and the anti-Notch1

antiserum we used is against the intracellular domain of the protein, it is difficult to completely rule out the possibility that the antiserum is also recognizing Notch1 proteins that are being synthesized and transported in the cytoplasm. However, in all the late metaphase to anaphase cortical progenitors we have examined (*n*=75), strong Notch1 immunoreactivity was always associated with the cell membrane and never filled the cytoplasmic space created by the breakdown of nuclear membranes during cell divisions.

In dividing cells at the ventricular surface, however, while m-Numb became asymmetrically localized to the apical or apical-lateral membrane (Fig. 7B-E; Zhong et al., 1996), the Notch1 immnuoreactivity remained distributed around the entire circumference of the cell membrane (Fig. 7G-M). Since apparently asymmetric immunostaining could arise from potential section artifacts, we randomly scored m-Numb or Notch1 staining in late prophase to anaphase ventricular cells (Table 1). Only staining covering less than 75% of the cell circumference was considered as being asymmetric.

We first used an antiserum against a transmembrane potassium channel, Kv1.4, as a control to test whether we could detect consistent asymmetric localization of its immunoreactivity at either apical or basal membrane of dividing cells. In E12.5 mouse neocortex sections, strong Kv1.4 immunoreactivity was detected both in the cortical plate (data not shown) and in a significant number of cells in the ventricular zone (Fig. 7F). In most of the dividing cells at the ventricular surface, Kv1.4 staining appeared to be symmetric (88.3%, *n*=86, late prophase to anaphase cells). A small percentage of the cells (11.7%) did show asymmetric Kv1.4 staining. However, the location of concentrated immunoreactivity appeared to be random (3.5% basal, 3.5% lateral and 4.7% apical). We then scored similar dividing cells for m-Numb staining with an anti-m-Numb antiserum. 41.7% of such dividing cells (*n*=94) exhibited an apical (or apical-lateral) crescent of m-Numb immunoreactivity. In these cells, m-Numb staining only covered less than half of the cell membrane with the basal or basal-lateral cell membrane exhibited no detectable m-Numb immunoreactivity. Only 3.2% of the cells showed basal (or basal-lateral) m-Numb localization and the rest showed little m-Numb staining throughout the entire circumference of the cell membrane, in contrast to the consistently detectable m-Numb around interphase cells. When similar E12.5 neocrotex sections were stained for Notch1, 93.4% of the dividing cells (*n*=106) exhibited symmetric Notch1 immunoreactivity around the cell membrane. The Notch1 staining in these cells was as intense as that in interphase cells of the ventricular zone. A small number of cells also showed localized Notch1 immunoreactivity (4.7% basal or basal-lateral and 1.9% lateral).

Table 1. Distribution of m-Numb, Notch1 and Kv1.4 immunoreactivity in dividing ventricular cells.

	%Asymmetric			
	Basal	Lateral	Apical	%Symmetric
Notch (*n*=106)	4.7	1.9	0	93.4
Numb (*n*=94)	3.2	0	41.7	55.1
Kv1.4 (*n*=86)	3.5	3.5	4.7	88.3

Only late prophase to anaphase cells at the ventricular surface were examined. The cells were randomly selected from at least three E12.5 mouse neocortex sections. Only staining covering less than 75% of the cell membrane was considered as being asymmetric. Apical and basal crescents also include apical-lateral and basal-lateral centered crescents, respectively.

Numblike can bind Notch1 directly

The ability of Nbl to mimic d-Numb misexpression phenotypes in *Drosophila* embyros suggests that Numb can interact with downstream effectors of d-Numb. Since d-Numb may function, at least partly, by causing a bias in the Notch mediated cell-cell interaction via direct physical interaction with Notch (Jan and Jan, 1995; Guo et al., 1996), we tested whether Numblike, like m-Numb, could also directly interact with the intracellular domain (ICD) of Notch1, a mammalian homologue of Notch (Fig. 8).

We first used the yeast two-hybrid assay (Bartel et al., 1993). We constructed two plasmids, one carrying a gene encoding a fusion protein of the LexA DNA-binding domain and Nbl and the other carrying a gene encoding a fusion protein of Notch1 ICD and the GAL4 transcription activation domain. As shown in Fig. 8A, cotransformation of both plasmids into a yeast reporter strain resulted in the detection of significant β-galactosidase reporter activity, whereas control experiments using one vector and one plasmid carrying a fusion gene had little effects (Fig. 8A).

We then performed a biochemical analysis as an independent means of verifying the Nbl-Notch1 interaction observed in the two-hybrid assay. We used a fusion protein of glutathione S-transferase (GST) and Notch1 ICD that was present in protein extracts containing an excessive amount of crude bacterial proteins. Mixing such extracts with an in vitro translated Nbl protein resulted in the specific co-purification of Nbl by glutathione agarose beads (Fig. 8B), but not the vast majority of bacterial proteins, further confirming that Nbl can bind to Notch1 directly.

DISCUSSION

We have reported here the cloning and characterization of *numblike*, a mammalian gene with sequence similarity to *d-numb* and *m-numb*. *nbl* encodes a cytoplasmic protein that, when expressed in *Drosophila*, is symmetrically distributed in dividing neural precursors and presumably partitioned into both daughter cells to cause the daughter cells to both adopt the fate normally determined by d-Numb. During mouse cortical neurogenesis, *nbl* is expressed in terminally differentiating neurons in the cortical plate, but not in progenitor cells within the ventricular zone where *m-numb* and *Notch1* are expressed. We have also found that, in dividing cortical progenitors, Notch1 appears to be symmetrically distributed around the membrane. We propose that an interplay between cell-intrinsic mechanisms (executed by *m-numb* and *numblike*) and cell-extrinsic mechanisms (mediated by *Notch*) may be involved in both progenitor cell proliferation and neuronal differentiation during mammalian cortical neurogenesis.

Asymmetric localization is not required for Numb to specify daughter cell fate

The high degree of sequence similarity between Nbl and d-Numb (Fig. 2) suggests that, like m-Numb (Zhong et al., 1996), Nbl may

mimic the function of d-Numb. Indeed, when expressed in *Drosophila numb* mutant embryos, Nbl allows SOP daughter cells to both adopt the fate normally determined by the presence of d-Numb (Fig. 4) (Rhyu et al., 1994). This is likely the result of Nbl being unable to localize asymmetrically in dividing neural precursors (Fig. 3) while still being able to interact with downstream effectors of d-Numb function.

When expressed in dividing neural precursors in *Drosophila*, m-Numb, the previously identified mammalian homologue of d-Numb, can be asymmetrically localized in a way similar to d-Numb (Zhong et al., 1996). Since Nbl and m-Numb share extensive sequence similarity throughout the protein, sequence comparison between these three Numb proteins may provide some clues to the requirement for asymmetric Numb localization.

numblike and *numb* may have overlapping but distinct functions during mammalian neurogenesis

The ability of both Nbl and m-Numb to allow daughter cells of *Drosophila* neural precursors to adopt the fate normally determined by the presence of d-Numb suggests that they have overlapping functions in mice. *nbl* and *m-numb* are both expressed in differentiating neurons outside the ventricular zone during development and in adult neurons throughout the nervous system (Fig. 6 and data not shown). Therefore, it is likely that *nbl* and *m-numb* have redundant functions in specifying and maintaining neuronal differentiation.

However, several lines of evidence suggest that *nbl* and *m-numb* also have distinct functions in mice. First, *nbl* and *m-numb* have different spatial and temporal expression patterns. *m-numb* is expressed in most tissues whereas *nbl* is highly enriched in the nervous system (Figs 5 and 6). Furthermore, in the developing nervous system where both genes are expressed, *nbl* mRNA is only expressed in differentiating neurons outside the ventricular zone, whereas *m-numb* mRNA is also expressed in dividing neural progenitors (Figs 6 and 7). Second, Nbl and m-Numb exhibit different subcellular localization. While m-Numb is a membrane associated protein like d-Numb (Fig. 3; Zhong et al., 1996), Nbl appears to be a cytoplasmic protein (Fig. 3). Third, when expressed in *Drosophila* neural precursors, m-Numb can localize asymmetrically (Zhong et al., 1996), whereas Nbl is unable to do so (Fig. 3). This suggests that, even if Nbl were expressed in mammalian neural progenitors, it would be unable to fully substitute for m-Numb (Zhong et al., 1996).

Distribution of mammalian Notch1 protein in dividing cortical progenitors

It has been recently reported that, in the ferret, the Notch1

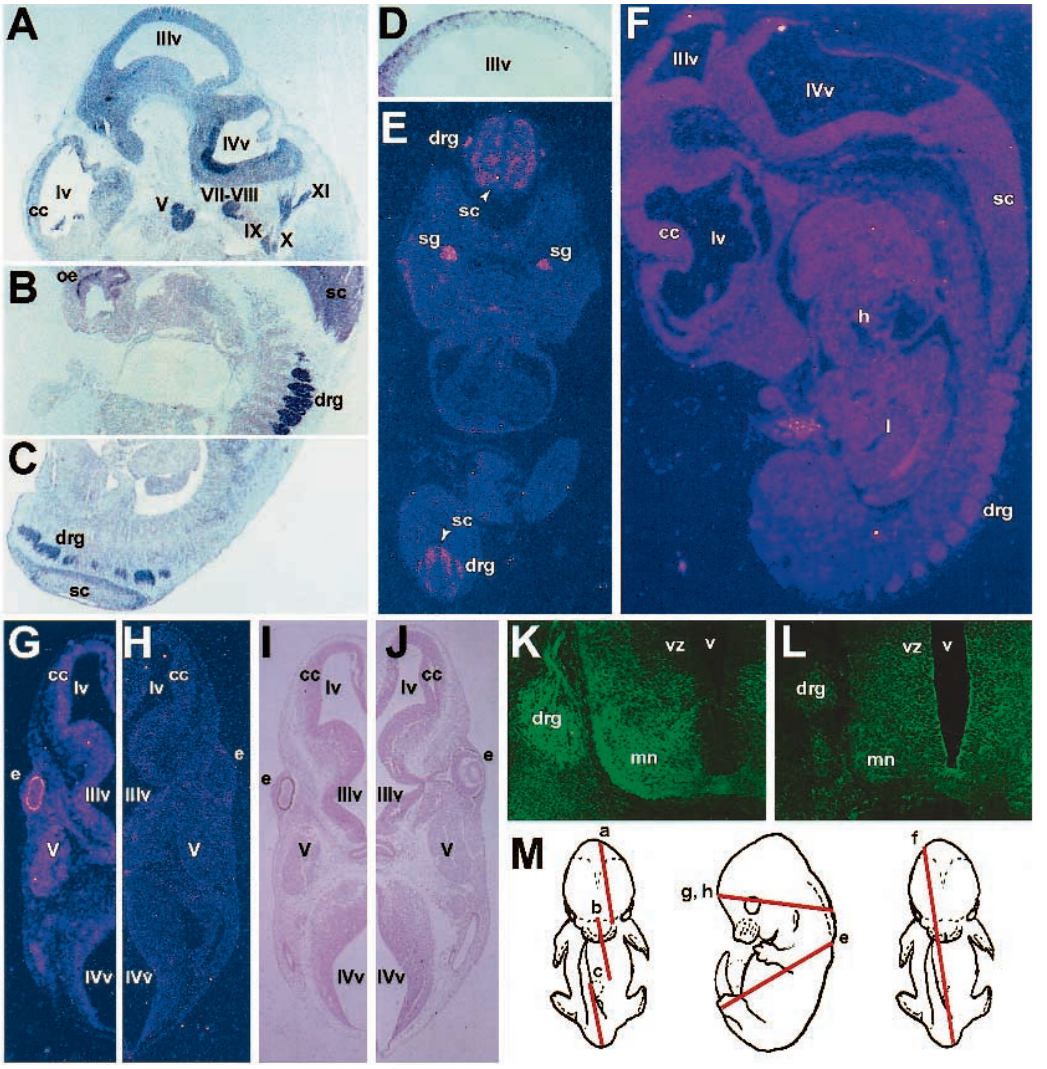

Fig. 6. Differential expression of *nbl* and *m-numb* during mouse embryogenesis. All sections are from E12.5 mouse embryos. lv, lateral ventricle; IIIv, third ventricle; IVv, fourth ventricle; V-XI, cranial sensory ganglia V to XI; cc, cerebral cortex; drg, dorsal root ganglia; e, eye; h, heart; l, liver; mn, motor neurons; oe, olfactory epithelia; sc, spinal cord; sg, sympathetic ganglia; v, ventricle; vz, ventricular zone. (A-E) *nbl* expression revealed by in situ hybridization. (A-D) DIG-labeled *nbl* antisense RNA probe, signals are dark blue; (E) ^{33}P-labeled *nbl* antisense RNA probe, signals are pseudo-colored pink. (F-J) *m-numb* expression revealed by in situ hybridization. (F,G) ^{33}P-labeled antisense RNA probe; (H) ^{33}P-labeled sense RNA probe as a control; (I,J) bright-field images of G and H, respectively. Note that *m-numb* is expressed in most embryonic structures (F,G). (K,L) Immunofluorescence staining of two spinal cord sections using antibodies against Nbl and m-Numb, respectively. (M) Respective section planes for images in A-H.

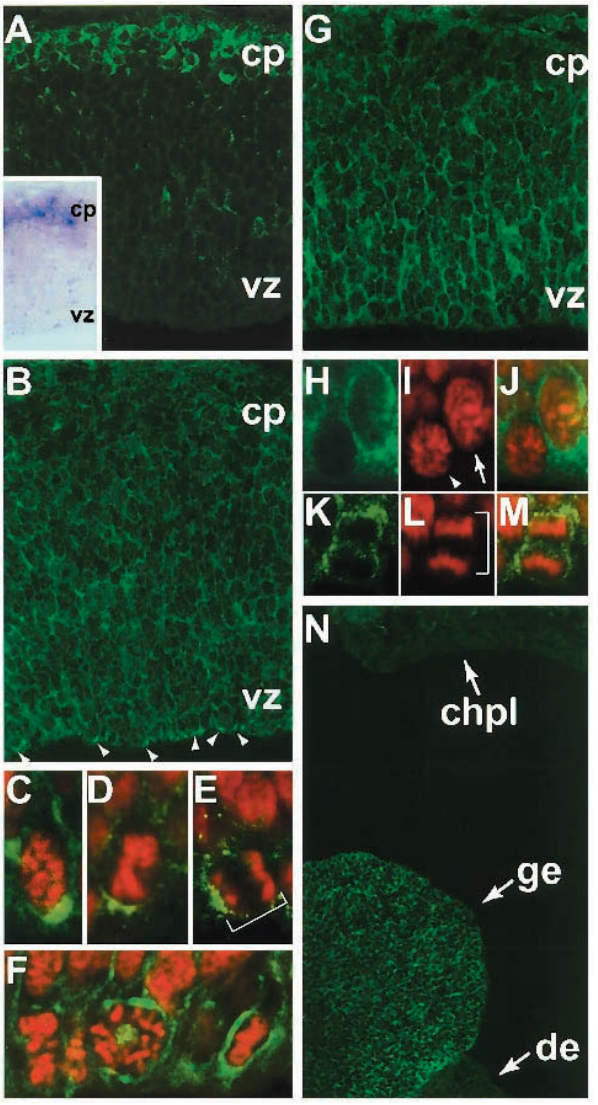

Fig. 7. Differential distribution of Numblike, m-Numb and Notch1 in the embryonic mouse neocortex. All sections are from E12.5. Immunoreactivities are revealed by immunofluorescence (in green). Red is propidium iodide chromosome staining. cp, cortical plate; chpl, choroid plexus; de, diencephalon; ge, ganglionic eminence; vz, ventricular zone. The ventricular surface is down (A-M). A, B and G have the same magnification, while C-F and H-M (at the ventricular surface) have a higher and N has a lower magnification. (A) Nbl. The insert is an in situ hybridization of *nbl* using DIG-labeled antisense *nbl* probes. Note that the low level of staining in the ventricular zone is likely due to cross-reaction of the antibody to m-Numb (see Materials and Methods). (B-E) m-Numb. Note the apical or apical lateral m-Numb crescents at the ventricular surface in B (arrowheads), C (a prophase cell), D (a metaphase cell) and E (an anaphase cell, bracket). The anaphase cell has a cleavage plane roughly 45° to the ventricular surface. (F) The immunoreactivity of a control antiserum against Kv1.4, a transmembrane potassium channel protein, in dividing cells at the ventricular surface. (G-N) Notch1 immunoreactivity in the neocortex (G-M) and part of the forebrain (N). H-M show ventricular cells at prophase (H-J, arrow), metaphase (H-J, arrowhead) and anaphase (K-M, bracket), as revealed by propidium iodide chromosome staining. The anaphase cell (bracket) has a cleavage plane roughly parallel to the ventricular surface. J and M are superimposed images of H-I and K-L, respectively.

A

LexA DNA-Binding Domain	Gal4 Activation Domain	β-galactosidase Activity
Vector	Vector	-
Numblike	Vector	-/+
Vector	NICD	-
Numblike	NICD	++

B

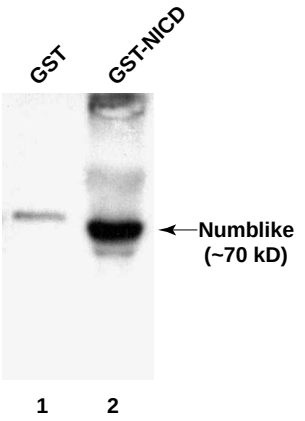

Fig. 8. Physical interaction of Numblike and Notch1. (A) The yeast two-hybrid assay of Nbl and Notch1 interaction. Left column, fusion proteins of LexA DNA-binding protein (vector) and Nbl; middle column, fusion proteins of Gal4 transcritpion activation domain (vector) and Notch1 ICD; right column, β-galactosidase activity of corresponding co-transformation experiments revealed by filter X-gal assay (Bartel et al., 1993). (B) Copurification of Nbl and Notch1 ICD. GST, glutathione S-transferase; GST-NICD, fusion protein of GST and Notch1 intracellular domain. Immunoblot analysis of Nbl in proteins purified by glutathione agarose beads in the presence of either GST alone (lane 1) or the GST-NICD fusion protein (lanes 2).

immunoreactivity is localized to the basal membrane of dividing cortical ventricular cells and can be differentially segregated to only the basal daughter cells (Chenn and McConnell, 1995). However, we have found that, in the great majority of neural progenitor cells in mice, Notch1 is distributed around the entire circumference of the cell membrane and segregated to both daughter cells. The anti-Notch1 antibody we used is a polyclonal antiserum raised against the intracellular domain of Notch1, which is highly conserved in other mammalian Notch proteins (Weinmaster et al., 1991, 1992; del Amo et al., 1993; Lardelli et al., 1994). In principle, it is possible that the Notch1 immunoreactivity we have observed may also be contributed to by Notch2 and Notch3, both of which are also expressed in the embryonic neocortex. On immunoblots, however, the antiserum fails to recognize in vitro synthesized Notch2 protein (G. Weinsmaster, unpublished observation). Furthermore, the staining pattern in the E12.5 embryonic brain appears to reflect the distribution of *Notch1* mRNA, but not

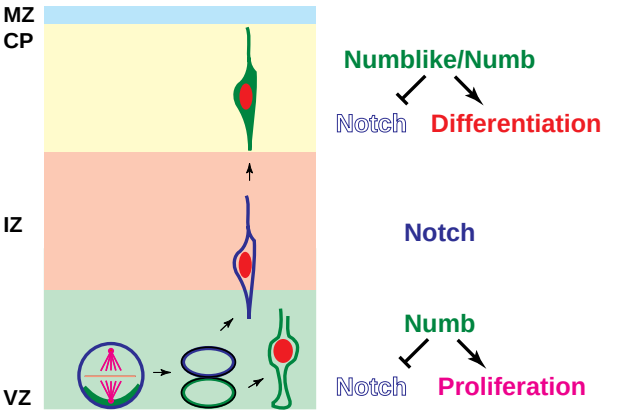

Fig. 9. A model for cortical neurogenesis with respect to m-Numb, Notch1 and Numblike activities. During a horizontal asymmetric cell division at the ventricular surface, the apical daughter cell inherits the majority of m-Numb which suppresses the Notch1 activity within the cell, thereby allowing it to remain as a progenitor in the ventricular zone (VZ). Its basal counterpart, however, inherits very little m-Numb and, as a result, has a relatively higher Notch1 activity that suppresses the progenitor cell fate. After migration through the intermediate zone (IZ) to the cortical plate (CP), the basal daughter cell expresses Nbl and m-Numb, which suppress the residual Notch1 activity and allow it to terminally differentiate into a neuron.

Notch2 or *Notch3* mRNA, as previously reported using in situ hybridization (Lardelli et al., 1994). For example, Notch1 immunoreactivity could not be detected in the choroid plexus, where *Notch2* mRNA is specifically expressed, nor in the diencephalon, where *Notch3* expression is significant (Fig. 7N).

Chenn and McConnell (1995) observed little Notch1 staining in most interphase cells of the ventricular zone or at the apical surface of dividing cells in the ferret. It is possible that the presence of a significant amount of Notch1 immunoreactivity throughout the entire circumference of dividing ventricular cells in mice prevented us from observing quantitative differences in the amount of Notch1 protein present in apical and basal cell surface. It is also possible that only specifically modified Notch1 protein that is undetectable by our antibody is localized in mice as in ferrets. A third possibility is that there are species differences in Notch1/Numb localization; ferrets may localize both Notch1 and Numb (or only Notch1) for its cortical neurogenesis.

Dynamic distribution of Nbl, m-Numb and Notch1 proteins during mammalian cortical neurogenesis

During mammalian cortical neurogenesis, cortical progenitors occupy the inner, ventricular zone, whereas newborn neurons migrate to the outer, cortical plate where they differentiate into various neurons (reviewed by Rakic, 1988; Caviness et al., 1995; McConnell, 1995). In the developing mouse neocortex, the *nbl* mRNA is only detected in cells at the cortical plate while Nbl immunoreactivity is also mainly observed in such cells. Therefore it is likely that *nbl* is only expressed by differentiating neurons at the cortical plate. Notch1, in contrast, is primarily expressed in proliferating cells of the ventricular zone, while m-Numb is expressed in all layers of the cortical neuroepithelium. Furthermore, in dividing cells at the ventricular surface, m-Numb is asymmetrically localized to the apical surface, whereas we have found that Notch1 remains symmetrically distributed around the membrane (Fig. 7).

It has been suggested that when a cortical progenitor divides horizontally with the cell cleavage plane parallel to the ventricular surface, it generates an apical daughter cell that remains as a progenitor and a basal daughter cell that leaves the ventricular zone for terminal differentiation (Chenn and McConnell, 1995). Such asymmetric divisions are, at least partly, responsible for cortical neuron production. It has also been suggested that, in *Drosophila*, d-Numb functions by physically interacting with the intracellular domain of Notch to cause a bias in Notch mediated cell-cell interactions (Jan and Jan, 1995; Guo et al., 1996). Furthermore, both m-Numb and Nbl can physically interact with the intracelluar domain of Notch1 (Zhong et al., 1996 and Fig. 8).

Based on these observations, we propose that the dynamic distribution of m-Numb, Notch1 and Nbl proteins provides a molecular basis for decisions concerning neural progenitor proliferation and neuronal differentiation during cortical neurogenesis (Fig. 9). During a horizontal cell division, Notch1 is partitioned into both daughter cells while m-Numb is segregated preferentially to the apical daughter cell. m-Numb suppresses the Notch1 activity in the apical daughter cell, thereby allowing it to remain as a progenitor (Zhong et al., 1996). The basal daughter cell, in the absence of inherited m-Numb, exhibits a relatively higher Notch1 activity and, as a result, is not a progenitor, nor does it become a fully differentiated neuron until it reaches the cortical plate (Chenn and McConnell, 1995). Once there, newly accumulated Nbl and m-Numb suppress the residual Notch1 activity in the cell and allow it to fully differentiate into a neuron.

Such a scenario for cortical neurogenesis is consistent with functions proposed for Numb and Notch in cell fate determination during *Drosophila* development. Numb functions not to specify one particular fate but rather to ensure that daughter cells of asymmetric divisions acquire distinct fates (Jan and Jan, 1995; Zhong et al., 1996). In SOP cell divisions during adult es organ formation, for example, d-Numb is involved in specifying the IIb daughter cell of SOP, as well as the socket daughter cell of the IIa cell and the neuron daughter cell of the IIb cell (Rhyu et al., 1994). Notch, however, may play a restrictive rather than instructive role in cell fate decisions (reviewed by Artavanis-Tsakonas et al., 1995). For instance, transient expression of an activated form of Notch does not permanently endow cells with particular fates. Once Notch signaling subsides, those cells are able to recover and develop properly or respond to later developmental cues (Fortini et al., 1993; Struhl et al., 1993). It is worth noting that, in our model, only one of the proteins, either m-Numb or Notch1, is required to be localized asymmetrically to provide a molecular basis for differential daughter cell fate specification, and that localization of both proteins to opposite poles of the cell would further ensure an asymmetric division.

Recently, Verdi et al. (1996) reported the immunostaining of m-Numb in mice. The m-Numb peptide used as an immunogen by Verdi et al. (1996) is derived from a conserved region between m-Numb and Nbl (14 out of 16 amino acid residues are identical to the corresponding portion of Nbl). A cross-reactivity of this antiserum to Nbl and m-Numb may account for their immunostaining of the developing mouse neural tube, showing stronger immunoreactivity in the mantal zone than in

the ventricular zone, as well as the symmetric cytoplasmic staining they oberved in developing DRG neuroblasts (Verdi et al., 1996).

It is likely that asymmetric cell divisions are not the only means for cortical neuron production, especially during late cortical neurogenesis when other mechanisms may also be involved (Kornack and Rakic, 1995; Reid et al., 1995; and reviewed by McConnell, 1995; Rakic, 1995). It is also likely that other factors are also required for various developmental decisions during cortical neurogenesis. Indeed, diffusible factors such as bFGF and NT3 have been shown to affect proliferation of neural progenitors and neuronal differentiation, respectively (Ghosh and Greenberg, 1995; Vicario-Abejon et al., 1995). Future experiments are required to test differential effects of *m-numb/nbl* and Notch functions, as well as how cell-intrinsic (mediated by *m-numb* and *nbl)* and cell-extrinsic mechanisms (mediated by Notch) are integrated during cortical neurogenesis.

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