Identification of New Human Mastermind Proteins Defines a Family That Consists of Positive Regulators for Notch Signaling*

Sey-En Lin‡‡, Toshinao Oyama‡‡, Takahiro Nagase‡, Kenichi Harigaya‡, and Motoo Kitagawa‡‡**

From the ‡Department of Molecular and Tumor Pathology, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan and the ¶Kazusa DNA Research Institute, 1532-3 Yana, Kisarazu, Chiba 292-0812, Japan

Mastermind (Mam) is one of the evolutionarily conserved elements of Notch signaling. Genetic analyses in Drosophila implicated it as an important positive regulator of the pathway. We show here identification of two new members of human Mam family (human Mastermind-2 (hMam-2) and human Mastermind-3 (hMam-3)), which retain characteristics similar to human Mastermind-1 (hMam-1) and Drosophila Mastermind. Both hMam-2 and hMam-3 stabilize and participate in the DNA-binding complex RBP-J/CBF-1 protein and the Notch intracellular domains that serve as intermediates of the signaling. Both hMam-2 and hMam-3 enhanced the activation of transcription from a target promoter by Notch signaling. However, we also show evidence that the activation of the target promoter by Notch3 and Notch4 is more efficiently potentiated by hMam-2 than by hMam-1 or -3. The multiplicity of Mam proteins in the mammalian system may help provide divergence to the strength of the Notch signals in different cell types.

Notch signaling is an evolutionarily conserved mechanism that mediates cell-cell communications required for cell fate decisions in metazoans (1, 2). In vertebrate systems, Notch signaling has been shown to mediate cell type specification in multiple tissues (3–8). Furthermore, abnormalities in Notch signaling were linked to human diseases such as neoplasia, stroke, syndromic intrahepatic cholestasis, an axial skeletal defect, and a congenital heart disease (9–13). In the vertebrates, four Notch genes are receptor type molecules that activate the target. Thus, the multiplicity of Mam proteins may help provide divergence to the strength of the Notch signals in various cell types.

EXPERIMENTAL PROCEDURES

Plasmids—Deletion mutants of hMam-2 and hMam-3 were constructed by digestions with appropriate restriction endonucleases, blunt ending by T4 DNA polymerase or S1 nuclease, and ligation. The 3′ end of the coding region of hMam-2 (11133) was fused to the coding region of green fluorescence protein (GFP) with double point mutations (GFP (P64L,S65T)) from pEGFP-C1 (Clontech) to give hMam-2-GFP. The 3′ end of the coding region of hMam-3 (11150) was fused to the sequence of GFP with single point mutation from pGreenLantern (In-
vitrogen) or Myc epitope from pcMV-Tag5 (Stratagene) to give hMam-3-GFP or hMam-3-Myc, respectively. The various hMam-2 and hMam-3 cDNAs were cloned into pEF-BOS (33) for expression in mammalian cells. Expression vectors for the intracellular domain of human Notch1 (hNotch1IC; pcDNA1/Amp-hNotch1IC) and RBP-J (pEF-RBP-J) were as described (25). Expression vectors for murine Notch1IC (residues Arg1715–Lys1853) and NotchIC (residues Gin1487–Asn1638) (pEF-BOS-neo-mNotch1 RAMIC and pEF-BOSneo-mNotch4 RAMIC) were also described (20). Expression vectors for murine Notch2IC (residues Ala1700–Ala2470) and Notch3IC (Ala1666–Ala2126) were constructed by inserting the respective cDNAs (34) into the pEF-BOSneo vector.

**Northern Blotting**—Multiple tissue Northern blot and multiple tissue Northern blot (Clontech) membranes that had been immobilized with polyadenylated RNAs from various human tissues were hybridized with 32P-labeled fragments from hMam-1 (2.4 kb; XhoI-KpnI), or hMam-3 (2.2 kb; SalI-BamHI) cDNA. Prehybridization, hybridization, and washing were done in stringent conditions under standard procedures. The washed membranes were analyzed with BAS2000 image analyzer (Fuji, Japan) without modification. After the analysis, the membranes were stripped off the radioactivity, hybridized again with human β-actin probe (Clontech), and analyzed as above. All of the experiments for the three probes were performed concomitantly.

**Antibody Production**—pGEX-hMam-1 (amino acids 1–344) was constructed by introducing the coding sequence of hMam-1 into pGEX-4T (Pharmacia Corp.). Glutathione S-transferase fusion protein was expressed in the BL21 strain of *Escherichia coli*. The rabbits were immunized by the purified protein with a standard procedure to raise antisera against hMam-1 protein. Immunoglobulin was purified with protein G column. Two of the antibody preparations (2-4 and 3-1) were cross-reacted with hMam-2 protein.

**Immunoprecipitation and Immunoblotting**—293T cells maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum were transfected with plasmids by the calcium phosphate method. Two days after the transfection, the protein was extracted from the cells with 20 μl Hepes-NaOH (pH 7.9) buffer containing 0.5% Nonidet P-40, 15% glycerol, 300 mM NaCl, 1 mM EDTA, 10 mM NaF, 1 mM benzamidine, and 0.5 mM sodium orthovanadate (25). The whole cell extracts were immunoprecipitated with an anti-Notch1 (Santa Cruz; sc-6014) or an anti-Posh (Santa Cruz; sc-8280) and protein G-Sepharose (Pharmacia Corp.). After separation by SDS-PAGE, the proteins were electrophoretically transferred onto polyvinylidene difluoride membranes (Bio-Rad). Primary antibodies used for blotting were anti-hMam-1 (2–4 and 3–1), anti-c-Myc (9E10), anti-Notch1 (sc-6014), anti-RBP-J (T6719) (35), anti-Posh (sc-8280), and anti-GFP (Santa Cruz; sc-8334). They were visualized with appropriate secondary antibodies conjugated with horseradish peroxidase, SuperSignal (Pierce), or Lumi-Light Plus (Roche Molecular Biochemicals) chemiluminescent substrate, and x-ray films. In some cases, the membranes were stripped and reblotted.

**Electrophoretic Mobility Shift Assay (EMSA)**—EMSA using transfected 293T cells were detailed in Ref. 25. Briefly, cells (2 × 105 cells/10-cm dish) were transfected with 5 μg each of the expression vectors for RBP-J, NotchIC, and Mam constructs or their empty counterparts. Total amount of the plasmid DNA was kept constant (15 μg). DNA-protein binding reactions were done by incubation of the whole cell extracts (20 μg, equivalent to the protein amount) in a solution (15 μl) containing 13 mM Hepes-NaOH (pH 7.9) buffer supplemented with 8% glycerol, 50 mM NaCl, 0.4 mM MgCl2, 0.5 mM dithiothreitol, 66.6 μg/ml poly(dI-dC):poly(dI-dC), and 33.3 μg/ml salmon sperm DNA for 15 min on ice, followed by an additional 30-min incubation with 32P-labeled synthetic oligonucleotide probe (0.1–0.2 μg, 4 × 105 cpm) at room temperature. The final concentration of the probe is 0.25–0.5 fmol/ml. Half of the mixture was loaded on polyacrylamide gels (5%) in 0.5× Tris-borate-EDTA buffer to separate the DNA-protein complexes. The complexes were detected by exposing the gels to x-ray films. For competition analysis to define sequence specificity of DNA binding, the molar excess of unlabelled double-stranded oligonucleotides was included in the binding reaction. The sequences of the oligonucleotides for the labeled probe were from the murine HES-1 gene that include the RBP-J-binding site (–91 to –56; HES-1 probe) (25). The competitors were the unlabelled HES-1 probe itself or dNTP probe that binds specifically to activated STAT1 and STAT3 (36, 37). In addition, the double-stranded oligonucleotides corresponding to the sequences were included in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. For transfection, the cells were seeded in 12-well dishes (0.3 × 105 cells/well). For assay with hNotch1IC, the cells were cotransfected with 0.3 μg of pHES-5uc reporter plasmid (23). 0.4 μg of pcDNA/Amp with or without hNotch1IC cDNA, 0.3 μg of pEF-BOS with or without Mam cDNAs, and 10 ng of Renilla luciferase internal control plasmid (pRL-CMV; Promega). For assay with the four murine NotchICs, the cells were cotransfected with 0.3 μg of pHER-5uc reporter plasmid, 0.3 μg of pEF-BOSneo with or without NotchIC cDNAs, 1 ng of pRL-CMV, and 0.4 μg of mixtures of pEF-BOS and the expression vectors for the three Mams in various ratio. The transient transfection was done using LipofectAMINE (Invitrogen). Two days after transfection, firefly and Renilla luciferase activities were determined using a dual luciferase assay kit (Promega) and a Turner Designs TD20/20 dual luminometer. Firefly luciferase activities were normalized with the Renilla luciferase control activities.

**Analysis of Subcellular Localization of GFP-tagged Proteins**—293T cells transfected with the expression vectors for the intracellular domain of human Notch1 (hNotch1IC; pcDNA1/Amp-hNotch1IC) and RBP-J (pEF-RBP-J) were as described (25). Expression vectors for murine Notch2IC (residues Ala1700–Ala2470) and Notch3IC (Ala1666–Ala2126) were constructed by inserting the respective cDNAs (34) into the pEF-BOSneo vector.

**RESULTS**

During a project to clone long human cDNAs, we have come across two cDNA sequences (KIAA1816 and KIAA1819) (38) that potentially encode proteins with similarity to hMam-1 (Fig. 1A). The overall identity between hMam-1 protein and each of the proteins encoded by these cDNAs is 30 and 20%, respectively (Table I). The arrangements of basic and acidic amino acid clusters in the Mam proteins including *Drosophila* Mam are well conserved, implying that their higher order structures are related (Fig. 1B). Based on the results reported below, we have tentatively named the protein encoded by KIAA1816 hMam-2 and that encoded by KIAA1819 hMam-3.

The expression of hMam-2 and hMam-3 mRNA in various human tissues was examined by Northern blotting analysis. For reference, hMam-1 mRNA was also analyzed in the same samples. As shown in Fig. 2, hMam-2 and hMam-3 mRNA was found as a single 7.9-kb transcript, and hMam-3 mRNA exists as a single 7.5-kb species. For hMam-3 mRNA, an additional 5.6-kb species was found in peripheral blood leukocytes and in placenta. Fig. 2 also shows that all three Mam mRNAs are expressed in many tissues with distinct patterns. Furthermore, it also indicates that multiple Mam mRNA species are expressed in a number of tissues.

We cloned each of the open reading frames of hMam-2 and hMam-3 cDNAs into a mammalian expression vector. When we blotted extracts of 293T cells transfected with these vectors, we found one of antisera raised against the N-terminal portion of hMam-1 protein cross-reacted with hMam-2. As shown in Fig. 3A, in addition to hMam-1 protein, which has an apparent molecular mass of 140 kDa, a single protein with an apparent molecular mass of 169 kDa was detected in the extract from the cells transfected with hMam-2. In contrast to hMam-2 protein, none of the anti-hMam-1 antisera reacted with hMam-3 protein (Fig. 3A). Thus, we attached a Myc tag to the C terminus of its coding region to identify the product of hMam-3 cDNA. Using an anti-Myc antibody, a single protein with an apparent molecular mass of 186 kDa was detected by Western blotting analysis of 293T cells transfected with this vector (Fig. 3B).

To test a hypothesis that these two proteins have functions similar to hMam-1, we transfected the expression vectors to 293T cells in combinations with vectors for the intracellular domain of human Notch1 (hNotch1IC) and a major form of mammalian CSL proteins, RBP-J/CBP-1 (39). Using EMSA, we analyzed binding activities in the extracts of the cells to a sequence from the HES-1 promoter, which has been shown to be essential for activation by the Notch1IC domain (22, 23, 25). As shown in Fig. 3C (lane 2), an extract from the cells transfected with RBP-J exhibited two specific bands that had been shown to contain RBP-J (25). Cotransfection of hNotch1IC with RBP-J induced another RBP-J/hNotch1IC complex that mi-
**Fig. 1.** Primary structure of Mam proteins. A, primary sequences of hMam-1 (GenBank™ accession number D83785), hMam-2 (AB058719), and hMam-3 (AB058722) polypeptides. Sequences were aligned with the ClustalW algorithm. Identical amino acids are in shaded boxes. Similar amino acids are in open boxes. The basic and acidic domains are in labeled boxes. B, arrangement of basic and acidic domains of *Drosophila* Mam (DMam), hMam-1, hMam-2, and hMam-3 proteins.
New Human Mastermind Proteins Augment Notch Signaling

**A**

![Amino Acid Sequence](image)

**B**

| Domain | Acidic Domain | Acidic Domain |
|--------|---------------|---------------|
| hMam   | 1 124 186 461 545 | 1548 1596     |
| hMam-1 | 1 127 214 295 | 991 1018      |
| hMam-2 | 1 65 128 272 353 | 1108 1133     |
| hMam-3 | 1 129 263 360 | 1124 1153     |

**FIG. 1—continued**
TABLE I
Homology between DMam, hMam-1, hMam-2, and hMam-3 polypeptides

Pairwise comparisons of percentages of amino acid identity among Mam proteins as calculated by the ClustalW algorithm. The values in parentheses represent similarities when allowing for conservative amino acid substitutions. Sequences analyzed were Drosophila Mam (DMam; GenBank™ accession number X54251), hMam-1 (D83785), hMam-2 (AB058719), and hMam-3 (AB058722).

|          | DMam | hMam-1 | hMam-2 |
|----------|------|--------|--------|
| hMam-1   | 15 (24) | 30 (45) |        |
| hMam-2   | 14 (25) | 20 (34) | 22 (38) |
| hMam-3   |       |        |        |

Fig. 2. Expression of Mam-1, Mam-2, and Mam-3 mRNAs in various human tissues. The hybridization with β-actin probe was presented as the RNA-loading controls. p. b. leukocytes, peripheral blood leukocytes.

Fig. 3D shows the analysis with the hMam-3 vector. Expression of hMam-3 with RBP-J and hNotch1IC again results in similar binding activities to the RBP-J/hNotch1IC/hMam-1 and RBP-J/hNotch1IC/hMam-2 transfection (Fig. 3D, lanes 4–6). Expression of hMam-3 without hNotch1IC also does not significantly alter the DNA binding activity of RBP-J or endogenous RBP-J-like factor (Fig. 3C, lanes 1, 2, 6, and 7).

To verify the DNA binding specificities of these complexes, we examined the effects of unlabeled competitors on the DNA binding reaction. As shown in Fig. 3E, the fast migrating complex in the vector-transfected extract and the two specific complexes in the RBP-J-transfected extracts were competed by molar excess of the unlabeled HES-1 probe but not by the same molar excess of a double-stranded oligonucleotide with unrelated sequence. Furthermore, all of the slowly migrating complexes in the RBP-J/hNotch1IC, RBP-J/hNotch1IC/hMam-1, RBP-J/hNotch1IC/hMam-2, and RBP-J/hNotch1IC/hMam-3 transfections showed significant competition by molar excess of the unlabeled probe.
transfected extracts were competed out by molar excess of the unlabeled HES-1 probe but not by the unrelated oligonucleotide (Fig. 3, F and G). These results indicate that RBP-J complex and all of the apparent multimeric complexes bind to the RBP-J site in the HES-1 promoter in a sequence-specific manner.

We next analyzed the complex formation by coimmunoprecipitation. This assay revealed that both hMam-2 and hMam-3 are, like hMam-1, specifically coprecipitated with hNotch1IC and RBP-J by an anti-Notch1 antibody but not by an anti-Posh antibody, which has been raised against the C-terminal portion of Posh (a downstream signaling molecule for Rac GTPase) protein (40) by the same manufacturer (Santa Cruz) using the same species (goat) as the anti-Notch1 antibody (Fig. 3, lanes 3–5 and 11–13) (25). These results indicate that both hMam-2 and hMam-3 associate with hNotch1IC and RBP-J in the absence of the binding site of DNA as shown for hMam-1 (25). This assay also disclosed that both hMam-2 and hMam-3 associate with hNotch1IC and RBP-J in the absence of the binding site of DNA as shown for hMam-1 (25). This result suggests that both hMam-2 and hMam-3 are, like hMam-1, specifically coprecipitated with hNotch1IC and RBP-J in the absence of the binding site of DNA as shown for hMam-1 (25).

The basic domains of the human Mam proteins are important for the complex formation. A, hMam-2 and its truncations containing the basic domain can associate with RBP-J and hNotch1IC within ternary complexes. The hMam-2 truncations, characterized in B, were tested for ability to form the ternary complex that is observed with full-length hMam-2. 293T cells were transfected with the indicated combinations of the expression vectors, and their extracts were analyzed for binding to the RBP-J element. The binding complexes migrate at a rate proportional to the length of the hMam-2 truncation. B, expression of the truncated forms of hMam-2 protein. Extracts of 293T cells transfected with the vectors for indicated proteins were blotted with the anti-hMam-1 antibody that cross-reacts hMam-2. To detect hMam-2 Met156stop protein, an x-ray film was exposed for longer period. C, sequence-specific binding of the complexes involving the truncated forms of hMam-2 protein. Analysis was performed as described for Fig. 3E, D, C-terminal truncations of hMam-3 can associate with RBP-J and hNotch1IC within ternary complexes. The hMam-3 truncations, characterized below, were tested for ability to form the ternary complex as described for A. The binding complexes migrate at a rate proportional to the length of the hMam-3 truncation. E, expression of the short forms of hMam-3 protein. Extracts of 293T cells transfected with the vectors for indicated proteins were blotted with the anti-c-Myc antibody. F, sequence-specific binding of the complexes involving the truncated forms of hMam-3 protein. Analysis was performed as described for Fig. 3E.
with the single protein species. The coimmunoprecipitation assays further reveal that expressions of all three human Mam proteins enhance the physical association of hNotch1IC and RBP-J (Fig. 2E, lanes 2–5) (25). These results indicate that hMam-2 and hMam-3 have characteristics very similar to hMam-1 in terms of complex formation with hNotch1IC and RBP-J.

We next examined the effect of hMam-2 and hMam-3 expression in comparison with that of hMam-1 in a transcriptional activation assay using the HES-5 promoter (25). As shown in Fig. 3I, the HES-5 promoter was activated by the expression of hNotch1IC alone but not by expression of hMam-1 alone. Co-expression of hNotch1IC and hMam-1 augmented the activation of the HES-5 promoter (25). Both hMam-2 and hMam-3 enhanced the hNotch1IC-induced activation of the HES-5 promoter to a similar extent to hMam-1, and these two new Mams did not activate the promoter in the absence of hNotch1IC (Fig. 3I). Because multiple Mam mRNAs are expressed in a number of tissues, we further examined whether the three Mam proteins exhibit synergism when expressed in combinations on the transactivation assay system. Fig. 3I shows, however, that no

|       | mNotch1 | mNotch2 | mNotch3 | mNotch4 |
|-------|---------|---------|---------|---------|
| mNotch1 | 40 (53) |         |         |         |
| mNotch2 | 40 (53) | 51 (63) |         |         |
| mNotch3 | 36 (49) | 45 (56) | 45 (58) |         |
| mNotch4 | 27 (37) | 30 (42) | 31 (42) | 32 (42) |

Pairwise comparisons of percentages of amino acid identity among Notch proteins as calculated by the ClustalW algorithm. The values in parentheses represent similarities when allowing for conservative amino acid substitutions. Sequences analyzed were Drosophila Notch (DNotch; GenBank™ accession number M16153), Notch1 (Z11886), Notch2 (D32210), Notch3 (NM_008716), and Notch4 (NM_010929).
combinations of the proteins elicited synergistic or combinato-
rial effects on the transactivation, if total amount of the trans-
fected vectors of the three was kept constant.

In hMam-1 protein, the N-terminal basic domain has been
shown to be essential for the complex formation with RBP-J
and Notch1IC (25, 32). We examined the effect of C-terminal
truncations of hMam-2 on the DNA-binding complexes involv-
ing RBP-J and hNotch1IC. As shown in Fig. 4A (lanes 3–5), all
of the truncations up to amino acid 156 reduced the complexes
involving RBP-J only and induced more slowly migrating com-
plexes whose mobility correlates with the molecular mass of
each truncation (Fig. 4B). Fig. 4C shows that the complexes
involving the hMam-2 truncations could be competed out by the
HES-1 oligonucleotide but not by the unrelated oligonucleotide,
verifying their binding specificity. These results support the
idea that the slowly migrating complexes contain hMam-2 pro-
tein. Furthermore, −156 amino acids from the N terminus are
sufficient to alter the mobility of the DNA-binding complexes,
and a deletion construct lacking amino acids 67–95 exhibits
virtually no activity on the complexes (Figs. 4A, lane 6, and 1B).
Thus, the N-terminal region of hMam-2 is necessary and suf-
cient to mediate the physical association. Similarly, C-termi-
nal truncations of hMam-3 up to amino acid 202 formed the
slowly migrating complexes whose mobility correlates with the
molecular mass of each truncation (Figs. 4, D and E, 3B, and 1B).
Again, all the slowly migrating complexes could be com-
peted out by the addition of the HES-1 oligonucleotide but not
by the unrelated oligonucleotide (Fig. 4F). These results indi-
cate that the N-terminal basic domain, a well conserved region
in the three proteins (Fig. 1A), is important for the complex
formation in all three.

It has been indicated that all four mammalian Notch pro-
teins undergo proteolytic processing during signaling and pro-
duce Notch1C-like molecular species as signaling intermedi-
ates (20, 21). We examined the complex formation between the
three Mam proteins with RBP-J and four murine NotchICs
that extend from juxtamembrane RAM domains to the C ter-
mini (20, 34). As shown in Fig. 5 (A–D), any combinations of
Mam and the NotchIC can be parts of complexes that resemble
those involving hNotch1IC (Fig. 3, C and D). We next investi-
gated whether expression of the three Mam proteins could
synergistically activate the transcription of the HES-5 pro-
moter with the four NotchICs. As shown in Fig. 5E, NotchIC-
induced activation was, in agreement with the experiment
shown in Fig. 3F, enhanced by coexpression of any of the three
Mams to similar degrees. Activation by Notch2IC was also
strongly activated by any of the three Mams to a comparable
extent (Fig. 5E). However, Notch3IC-induced activation was
not efficiently up-regulated by either hMam-1 or hMam-3. In
contrast, hMam-2 showed significant augmentation of the
Notch3IC-induced activation (Fig. 5E). Notch4IC-induced activa-
tion was relatively resistant to up-regulation by Mams (Fig.
5E). Only a high dose of hMam-2 showed significant augmenta-
tion (Fig. 5E). These results indicate that hMam-2 has a
unique role for the Notch3- and Notch4-mediated signaling.

Finally, to obtain an insight into subcellular localization of
hMam-2 and hMam-3, we attached GFP tags to the C termini
of their coding regions cloned in the mammalian expression
vector. When these vectors were transfected into 293T cells,
each produced a single protein with an apparent molecular
mass of 200 kDa as detected by Western blotting analysis with
an anti-GFP antibody (Fig. 6A). Because the tag attached to
hMam-2 is larger in molecular mass, it is consistent that the
fusion proteins were presented as the similarly sized bands.
When the transfected cells were analyzed with a confocal laser
scanning microscopy, both of the proteins were found as the
nuclear dots (Fig. 6B). These results suggest that as is the case
for hMam-1, both hMam-2 and hMam-3 are nuclear protein
and may localize in nuclear bodies (25, 32).

DISCUSSION

We have described the identification of two new human Mam
proteins that possess characteristics similar to hMam-1 and
Drosophila Mam (25). Both hMam-2 and hMam-3 contribute
to the formation of a ternary complex along with a CSL protein
and IC domain of the Notch receptors, and this complex asso-
ciates with an HES promoter sequence. The expression of
hMam-2 and hMam-3 augments Notch pathway-mediated ac-
tivation of the HES target depending on its context. The reli-
ance on the N-terminal basic domain for the complex formation
is also a conserved feature of the proteins.

It may be worth noting that Drosophila Mam, along with
hNotch1IC and RBP-J, forms a heterologous ternary complex
that cannot bind to the HES promoter.2 Furthermore, Drosoph-
ila Mam cannot augment the transcriptional transactivation
induced by hNotch1IC.3 Thus, the complexes that result from
promiscuous interaction between the mammalian versions of
Mam proteins and NotchICs seem to be evolved under func-
ional restraint and may be significant.

As paralogous proteins in a mammalian species, the three
Mams, especially hMam-3, seem to be significantly diverged
from each other in their primary structures compared with the
other groups of paralogues (Table I). For instance, one class of
the association partners of Mam, Notch proteins show 30–51% identities in pairwise comparisons among the mammalian se-
quences (Table II). One group of the mammalian ligands for
Notch, Dll1, Dll3, and Dll4 proteins exhibit 51–51% identity
(41). Another group of the Notch ligands, Jagged1 and 2 exhibit
54% identity in a mammalian species (42). Three mammalian
Fringe proteins that are glycosyltransferases that modify the
ligand interaction of Notch (43, 44) exhibit 47–64% identity
(45). Thus, one may feel it unexpected that the three mamma-
lian Mam proteins manifest such similar characteristics in
their function.

It is still to be elucidated why the mammalian system re-
quires multiple Mam species. In this sense, the preferences
that are shown by some NotchICs to Mam proteins as interact-
ing partners for the transactivation (Fig. 5E) may be signifi-
cant. Because the multiple Notch species have unique roles in
the mammalian systems (15–18), the plurality of Mam proteins
may have a role in the system providing divergence to the
strength of the Notch signals in various cell types in the mam-
malian systems.

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