

Notch Signalling Under Maternal-to-Zygotic Transition

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ABSTRACT

The development of all animal embryos is initially directed by the gene products supplied by their mothers. With the progression of embryogenesis, the embryo's genome is activated to command subsequent developments. This transition, which has been studied in many model animals, is referred to as the Maternal-to-Zygotic Transition (MZT). In many organisms, including flies, nematodes, and sea urchins, genes involved in Notch signaling are extensively influenced by the MZT. This signaling pathway is highly conserved across metazoans; moreover, it regulates various developmental processes. Notch signaling defects are commonly associated with various human diseases. The maternal contribution of its factors was first discovered in flies. Subsequently, several genes were identified from mutant embryos with a phenotype similar to *Notch* mutants only upon the removal of the maternal contributions. Studies on these maternal genes have revealed various novel steps in the cascade of Notch signal transduction. Among these genes, *pecanex* and *almondex* have been functionally characterized in recent studies. Therefore, in this review, we will focus on the roles of these two maternal genes in Notch signaling and discuss future research directions on its maternal function.

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Introduction

Cell signaling plays a pivotal role in the development and homeostasis of multicellular organisms. During recent decades, molecular mechanisms underlying various cell signaling pathways, such as the Notch signaling pathway discussed in this review, have been extensively studied. Because the components of these cell signaling pathways are mostly encoded in genomes, genetics has been a powerful approach for identifying and characterizing them. The phenotypes observed upon the mutation of these genes have been particularly informative, allowing researchers to dissect the cascades of these cell signaling pathways.

Genetic trait inheritance is commonly governed by Mendel's laws. However, in some cases, these traits are solely determined by the mother's genotype regardless of the offspring's genome. Such maternal effects were first identified in studies on genes that control development. For example, the coiling polarity of the pond snail *Lymnaea* is determined by maternal effects [1,2]. The shells of these snails show dextral (right-handed coiling) or sinistral (left-handed coiling) chirality; dextral

and sinistral mutants are dominant and recessive, respectively [1,2]. The shell's coiling direction is solely determined by the mother's genotype [1,2]. For example, a mother snail homozygous for the sinistral gene always bears sinistral offspring regardless of her mate's genotype (or the offspring accordingly). This observation indicates that the coiling polarity of the shell of *Lymnaea* is determined by maternal effects.

In *Drosophila*'s early embryogenesis, the roles of maternal gene functions have been understood. For example, a fly embryo's anterior-posterior polarity is initially defined by the concentration gradient of a transcription factor called Bicoid, whose concentration descends from the embryo's anterior end towards the posterior end. The Bicoid protein in the egg is produced from the *bicoid* mRNA provided by the mother. Hence, in the *bicoid* mutant offspring derived from homozygous mutant mothers, both the anterior and posterior ends develop as tails due to a failure in the anterior identity specification [3].

The events of early embryogenesis in flies, including polarity formation, often depend on the maternal functions of genes because these

events occur before the activation of the zygotic genes. Early embryos are equipped with mRNAs supplied to the egg by the mother during oogenesis. These maternal mRNAs gradually degrade as embryonic development proceeds. More importantly, various cell signaling pathways play crucial roles in early embryogenesis when maternal mRNAs or proteins predominantly function. Therefore, such maternal functions of genes are closely related to various cell signaling pathways responsible for early embryogenesis. However, it is time-consuming to remove each gene's maternal contribution to study the corresponding maternal effect, even when using genetic model animals such as *Drosophila*. Thus, the maternal functions of various genes involved in cell signaling pathways are still elusive. Nevertheless, the persistent efforts of many laboratories have revealed the essential roles of some of such maternal genes in cell signaling pathways required for early embryogenesis [4–8]. This review describes the maternal functions of the genes that contribute to early embryogenesis and discusses the recent findings on the roles of maternal genes in the Notch signaling pathway.

Maternal-to-Zygotic transition

Generally speaking, the embryogenesis of animals is initially directed by maternal gene products. In *Drosophila*, we know that approximately 55–65% of genes show a maternal contribution [9–11]. The functions and activities of maternal mRNAs are post-transcriptionally regulated through multiple mechanism layers. For example, the specific localization, translation efficiency, and degradation of maternal mRNAs are controlled by cis-acting elements within the maternal mRNAs and factors binding to them. Upon the depletion of maternal mRNAs, the transcripts from the zygotic genome start coordinating the embryonic development. Moreover, the ratio of maternal-to-zygotic mRNA amount is reversed during the early embryogenesis of various organisms (Figure 1). This phenomenon is generally called the Maternal-to-Zygotic Transition (MZT) [12]. MZT and its underlying mechanisms are commonly observed among metazoans [13–16]. However, there is a difference in MZT timing among species because

the half-lives of maternal mRNAs and the initiation of zygotic transcription differ among organisms (Figure 1). Hence, it is generally important to understand how zygotic genes, which are initially silent, are activated during the MZT. This activation is designated as Zygotic Genome Activation (ZGA).

Drosophila's early embryo is a syncytial blastoderm where nuclear divisions synchronously take place; these divisions are called Nuclear Cycles (NCs) (Figure 1). Early nuclear cycles that comprise the first hour of embryogenesis (NCs 1–8) take only 8 min each, which is too short to allow any zygotic transcription [17]. The gradual prolongation of NCs to 20 min by NC 13 triggers the low level of ZGA. In NC 14, the ZGA's bulk occurs, initiating the embryo's cellularization, and lasts for at least 70 min depending on the cell types; this is because cell divisions are no longer synchronous [18]. Simultaneously, a large part of the maternal RNA starts degrading. This is because maternal RNA degradation needs zygotic factors, like miRNAs, even though there is an earlier ongoing RNA degradation by a maternal pathway as well [19]. Recently, the dynamic transition of mRNA species coupled with the MZT has been revealed by genome-wide analyses [20–22].

Drosophila zelda (*zld*), which encodes a maternal transcription factor, was identified as a master regulator of ZGA [23]. The translation of maternally deposited *zld* mRNA is induced soon after fertilization and peaks upon the ZGA's occurrence [23–25]. Zld binds to a class of DNA-sequence motifs that are enriched in the cis-regulatory elements of genes activated in ZGA [26]. The enhancer regions of early expressed zygotic genes have an intrinsically high nucleosome barrier, and Zld overcomes it via the local depletion of nucleosomes [27,28]. Consequently, Zld establishes or maintains regions of open chromatin, thereby potentiating the binding of other transcription factors that promote the MZT. In concert with other transcription and chromosomal factors, Zld establishes a three-dimensional, high-order conformation that assures the expression of zygotic genes.

In this review, we discuss the components of maternally provided Notch signaling that have been extensively studied in *Drosophila*. Since Notch signaling acts through direct cell-cell

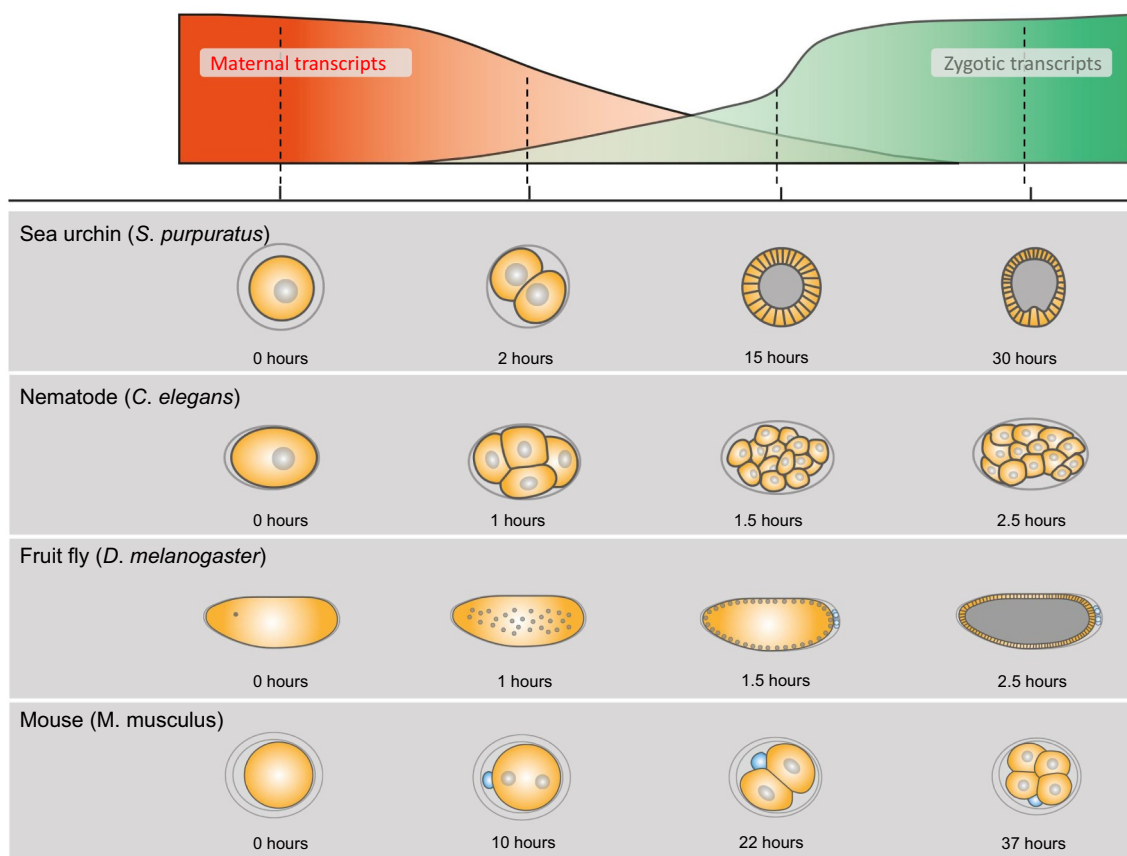


Figure 1. Clearance of Maternal Transcripts and Production of Zygotic Transcripts during the MZT in Various Animals. Schematic diagrams of the MZT in model organisms, including the sea urchin (*S. purpuratus*), nematode (*C. elegans*), fruit fly (*D. melanogaster*), and mouse (*M. musculus*). The maternal transcripts (orange curve) gradually degraded in proportion to the increase of zygotic transcripts (green curve). Each phase's length and the MZT's timing differ among species. The embryonic stages (schemas and hours after the development's commencement) of each animal are shown, corresponding to the MZT's phases.

interactions, the initiation of its functions needs to be coupled with the cellularization of the syncytial blastoderm (Figure 1). This idea is generally applicable to other cell signaling pathways mediated by extracellular signaling molecules and membrane receptors. The cellularization proceeds during the MZT, and a cellular blastoderm is formed in embryos around 2.5 hours post fertilization when ZGA is ongoing (Figure 1) [29]. Thus, shortly after the MZT, various cell signaling pathways activate the zygotic expression of their target genes. Considering the timing of such target-gene activation, we can infer that the components of these cell signaling pathways should be prepared before the MZT. *Drosophila* Notch signaling is no exception because it regulates the differentiation of mesectoderm, which occurs immediately after the MZT [30,31]. Studies have shown that various genes composing the Notch pathway have

a maternal contribution that supports early embryogenesis. However, how these maternal genes execute their functions in the Notch signaling during and after MZT remains unclear.

The Notch signaling pathway

The *Drosophila* Notch receptor is a transmembrane protein with 36 epidermal growth factor-like repeats in its extracellular domain [32] (Figure 2a). During the Notch protein's maturation, its extracellular domain is cleaved by the Furin protease (S1 cleavage) within the Golgi apparatus [33–35]. The S1-cleaved Notch protein is then reassembled and moved to the cell's surface [33–35]. At the surface, the Notch binds to its transmembrane ligand, Delta or Serrate, whose endocytosis induces a mechanical pulling force acting on the Notch's extracellular

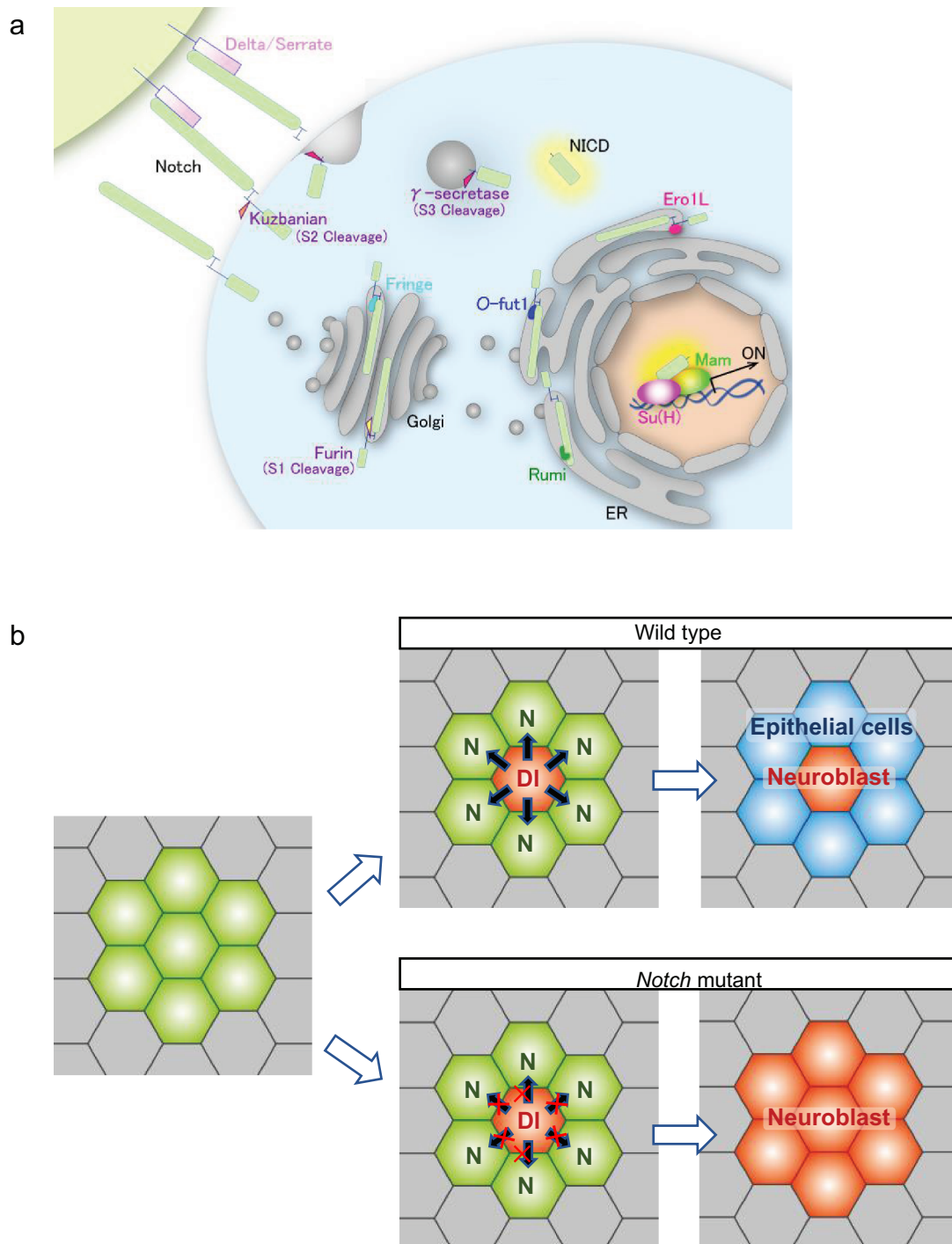


Figure 2. The Notch Signaling Pathway and its Function in Lateral Inhibition. (a) The Notch receptor (green) is produced in the Endoplasmic Reticulum (ER) and matured during transportation from the ER to the Golgi. The Notch is processed by a furin-like protease within the Golgi (S1 cleavage) and then migrates to the cell's surface. Additionally, in the ER and the Golgi, the Notch is glycosylated by *O*-fucosyltransferase1 (*O*-fut1) and Fringe, respectively. After binding the Notch to the ligands (Delta or Serrate, shown in pink), two proteolytic cleavages (S2 and S3 cleavages) are induced. The S2 cleavage is catalyzed by Kuzbanian/ADAM10, consequently removing the Notch extracellular domain. The S3 cleavage is catalyzed by γ -secretase, which occurs in the transmembrane domain of Notch and releases the Notch Intracellular Domain (NICD) from the cell membrane. Consequently, the NICD translocates to the nucleus where it forms a complex with the Mastermind (Mam) and Suppressor of Hairless [Su(H)] and activates the transcription of various target genes. (b) Notch signaling is involved in lateral inhibition and its failure results in a neurogenic phenotype. In *Drosophila* neuroectoderm, a neuroblast (orange) is sorted out from cells composing a proneural cluster (green) through lateral inhibition controlled by Notch signaling. Initially, proneural cluster cells have equivalent potential to choose fate of the neuroblast (left panel). However, in the wild type (upper), once one cell in a proneural cluster starts to acquire the neuroblast's fate, it begins to express *Delta* (*DI*) which activates the Notch signaling in the adjacent cells (middle). Consequently, Notch signaling prevents adjacent cells from choosing their neuroblast fates, making them epidermoblasts (blue) (left). In the *Notch* mutant (lower), *Delta* (*DI*) is unable to activate Notch signaling in the adjacent cells, which consequently fails to suppress the neuroblast fate (middle). Therefore, all adjacent cells differentiate into neuroblasts (orange) (right).

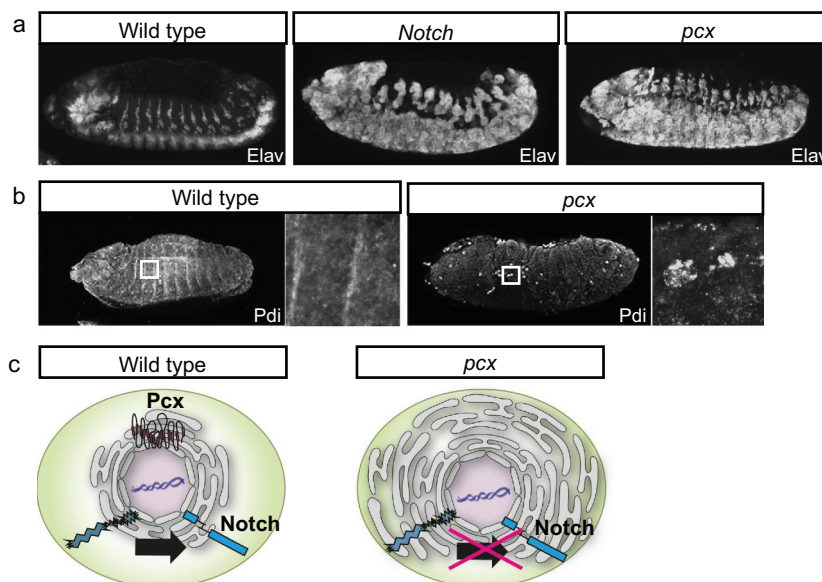


Figure 3. Pcx Promotes the Notch Signaling by Modulating the Endoplasmic Reticulum's (ER) Physiology. (a) This figure demonstrates the lateral views of the wild-type (left), the *Notch* mutant (middle), and *pcx* maternal/zygotic null embryos (right) at stages 13–14. Neurons are visualized by anti-Elav antibody staining (white). (b) The lateral views of the wild-type (left, wild type) and *pcx* maternal/zygotic null embryos (right, *pcx*) at stages 13–14. The ER is visualized by anti-Pdi antibody staining (white). Right insets show high magnifications of the regions indicated by white boxes in the left panels. (c) Schematics showing the ER structure of cells of the wild type (left, wild type) and the *pcx* maternal/zygotic null (right, *pcx*). The Pcx localizes to the ER where the Notch is properly matured in wild-type cells (left). However, the ER is enlarged and becomes defective in the absence of Pcx (right).

domain [36,37]. The pulling force induces a conformational change in the Notch's extracellular domain, thereby making the protein susceptible to the second cleavage by Kuzbanian/ADAM10 (S2 cleavage). This leads to the extracellular domain's removal [38,39]. Subsequently, the membrane-tethered form of the Notch intracellular domain (Notch Extracellular Truncation, NEXT) is cleaved within its transmembrane domain by γ -secretase (S3 cleavage), whereby the Notch Intracellular Domain (NICD) is released from the plasma membrane [40]. The NICD then translocates to the nucleus and activates the transcription of target genes [41,42].

Drosophila Notch signaling plays several important roles in development and homeostasis. The best-known role of Notch signaling during early development is 'lateral inhibition', which prevents the signal-receiving cells in the neuroectoderm from choosing the neuroblast fate [43] (Figure 2b). Therefore, Notch signaling disruption impairs this lateral inhibition and induces neural hyperplasia at the expense of epidermal cells, which is designated as a neurogenic phenotype [43] (Figure 3a). Thus, genes whose mutants show

neurogenic phenotypes are referred to as neurogenic genes. Since most of the genes encoding the components of Notch signaling are essential for lateral inhibition, Lehmann et al. (1983) first identified these genes based on the neurogenic phenotypes observed in the corresponding mutants [44].

The Maternal Members of Genes Composing the Notch Signaling Pathway in *Drosophila*

Based on the neurogenic phenotypes in embryos, genetic studies have identified five genes as components of Notch signaling: *Notch*, *big brain (bib)*, *mastermind (mam)*, *neuralized (neur)*, and *Delta (Dl)* [45]. Since all these mutants show zygotic neurogenic phenotypes, these genes are referred to as zygotic neurogenic genes. However, the zygotic *Notch* mutant's neurogenic phenotype is weaker than those of the zygotic *mam* mutant, suggesting that *Notch*, but not *mam*, has a maternal contribution [4]. This idea has been confirmed by the observation that homozygous *Notch* or *mam* mutants lacking respective maternal contributions show similar neurogenic phenotypes [4].

Furthermore, various laboratories have identified genes that showed a neurogenic phenotype in embryos lacking maternal and zygotic functions even though their zygotic mutants did not demonstrate a neurogenic phenotype. These genes are designated as ‘maternal neurogenic genes’ and they include *Suppressor of Hairless* [*Su(H)*], *O-fucosyltransferase1* (*O-fut1*), *Presenilin* (*Psn*), *anterior pharynx defective 1* (*aph-1*), *Nicastrin* (*Nct*), *pecanex* (*pcx*), *almondex* (*amx*), *biscotti* (*bisc*), and *amaretto* (*amrt*) [42,46–52]. Thus, their maternally deposited mRNAs and/or translated proteins are maintained after the MZT.

Among them, *Su(H)*, *O-fut1*, *Psn*, *aph-1*, and *Nct* are required for Notch signaling maternally, and these genes also function zygotically at later embryonic, larval, pupal and adult stages (maternal and zygotic class), showing that they encode its general components. Since these genes have zygotic functions in Notch signaling, the zygotic mutant of *Su(H)*, for example, shows phenotypes that are found in the zygotic class of neurogenic genes, such as *Notch*, *Dl*, and *fringe*. At the late embryonic stage of the *Su(H)* zygotic mutant, the proventriculus does not properly form, as in *Notch* zygotic mutant embryos [53]. This may be due to the depletion of the maternal mRNA of *Su(H)* by late embryogenesis. The analyses of the maternal and zygotic class neurogenic genes were crucial for our understanding of the signal transduction pathway of Notch signaling. Notably, the basic signal transduction cascade in the Notch signaling pathway was revealed via studies on these genes. On the other hand, *pcx*, *amx*, *bisc*, and *amrt* seem to be purely maternally required (purely maternal class) because their zygotic mutants do not show phenotypes related to the ablation of Notch signaling even at late stages of development [50–52]. This may suggest that the genes of the purely maternal class are specifically required for embryonic Notch signaling. However, it is difficult to exclude their roles in post-embryonic Notch signaling because their maternal products, likely proteins, may persist beyond the MZT and potentially until adulthood. This could compensate for the absence of zygotic gene functions in post-embryonic Notch signaling.

Our group has contributed to the analyses of several maternal neurogenic genes, including

O-fut1, *pcx*, and *amx* [46,54–57]. Important progress has been made in the understanding of *pcx* and *amx* functions, and the roles of these two maternal neurogenic genes in Notch signaling are discussed below.

pecanex

Drosophila pecanex (*pcx*) was identified as a mutant fly line with recessive female sterility [50]. Namely, homozygous *pcx* females survive until adulthood and oviposit fertilized eggs, whereas embryos deposited from them stop their development and demonstrate neural hyperplasia (Figure 3a). Embryos that are homozygous for *pcx* and lack maternal *pcx* show neural hyperplasia reminiscent of a neurogenic phenotype found in *Notch* mutant embryos (Figure 3a). Furthermore, the maternal neurogenic phenotype of *pcx* was paternally rescued [55,58]. Therefore, it was proposed that *pcx* is a component of the Notch signaling pathway. However, homozygous *pcx* females, with their maternal contribution, do not show detectable developmental defects. Moreover, the contribution of *pcx* to any context of Notch signaling other than embryonic neurogenesis has not been observed [55]. Hence, it is not clear whether *pcx* plays any role in postembryonic Notch signaling. *pcx* encodes a putative sixteen-pass transmembrane protein composed of 3,413 or 3,417 amino acids and is conserved from *Drosophila* to humans [58,59]. In mammals, four tissue-specifically expressed *pcx* paralogs (*Pcnx1*, *Pcmx2*, *Pcnx3*, and *Pcnx4*) have been identified, although their roles in Notch signaling remain unknown [60].

To deduce the step where *pcx* functions in the Notch signaling cascade, epistasis analyses between *pcx* and various mutant forms of *Notch* have been conducted [55]. The neurogenic phenotypes of the *pcx* maternal and zygotic null are suppressed by the overexpression of NEXT or a nuclear form of the activated Notch (NICD) [55]. These results suggest that *pcx* may act upstream of the S2 and S3 cleavages of Notch. In addition to the epistasis analysis, the cell biological functions of *pcx* have been studied. Embryos homozygous for *pcx* and lacking their maternal contributions show Endoplasmic Reticulum (ER) enlargement [55] (Figure 3b). However, *Notch* mutant embryos do not demonstrate such ER defects, suggesting that

the defects associated with the absence of *pcx* may lie upstream of the defects in Notch signaling [55]. This idea is also supplemented by a finding suggesting that *pcx* is an ER-resident protein [55]. Thus, *pcx* has been speculated to play a crucial role in the ER that is essential for normal Notch signaling activation (Figure 3c).

To analyse a potential function of *pcx* in the ER, ER physiology was modulated in the *pcx* heterozygote lacking its maternal contribution (*pcx* maternal/zygotic null). Unfolded Protein Response (UPR) is a cellular response against ER stress induced by accumulating unfolded proteins in the ER [61]. UPR suppresses the cell's stress levels by reducing the unfolded proteins [61]. The Xbp1 pathway is a major UPR cascade that activates the transcription of genes, encoding chaperone proteins that facilitate protein folding [62,63]. Upregulating the Xbp1 pathway by overexpressing an activated form of *Xbp1* in *pcx* homozygous embryos lacking their maternal contribution rescues their neurogenic phenotype. This observation suggests that the downstream events of the Xbp1 pathway may suppress the ER's physiological defect. These events include protein synthesis attenuation, enhancement of misfolded ER protein degradation, and induction of genes encoding various chaperones. However, at this point, the way the Xbp1 pathway compensates for the ER defects caused by the absence of the *pcx* function is still unclear.

To identify genes cooperatively functioning with *pcx*, a genetic screen was conducted, which involved a cold-sensitive lethality of the *pcx* maternal/zygotic null [56]. Various gene components of Notch signaling, such as *Delta*, *bib*, and *neur*, were identified as enhancers and suppressors of lethality, suggesting that the screen fulfilled its purpose. As a dominant suppressor, *N-ethylmaleimide-sensitive factor 2* (*Nsf2*), encoding a key regulator of vesicular fusion, was isolated from the screen [56]. *NSF* is known to control the cohesion of ER [64,65]. Considering that the loss of the *pcx* function induces ER enlargement, *Nsf2* and *pcx* may be antagonistic in the homeostasis of ER morphology [56].

almondex

In *Drosophila*, *amx* was identified as a maternal neurogenic gene because fertilized eggs obtained

from *amx* homozygous mothers showed strong neurogenic phenotypes [44,45,51,66] (Figure 4a). Therefore, *amx* is considered a component of the Notch signaling pathway. In the *Drosophila* genome, three paralogs of *amx* exist, namely *biscotti* (*bisc*), *amx*, and *amaretto* (*amrt*) [52]. A study revealed that all three are maternal neurogenic genes, implying that each of them plays maternal roles that are essential for Notch signaling [52]. However, triple homozygotes of *bisc*, *amx*, and *amrt* are viable due to the maternal contribution of the corresponding proteins. These mutants do not show detectable phenotypes in development until adulthood, demonstrating that *amx* paralogs have no zygotic role in the postembryonic development in *Drosophila* [52]. The *amx* family genes are evolutionarily conserved, and their mammalian orthologs are designated as the TM2 domain (TM2D) containing protein genes [67,68]. *bisc*, *amx*, and *amrt* correspond to mammalian orthologs: *TM2D1*, *TM2D2*, and *TM2D3*, respectively [52]. These *amx* family genes encode predicted double-pass transmembrane proteins with a conserved DRF motif and a TM2 domain [68]. Their structure and functions are conserved between *Drosophila* and mammalian orthologs because *TM2D3* can rescue the neurogenic phenotype of *Drosophila amx* mutant embryos [69].

The *Drosophila* model led to the development of an understanding of the *amx* gene's functions. Genetic epistasis between *amx* and *Notch* provided important insights for understanding where *amx* is required in the Notch signaling cascade [68]. The overexpression of a full-length Notch or NEXT fails to suppress the neurogenic phenotype in embryos that are homozygous for *amx* and devoid of the corresponding maternal contribution. On the contrary, the NICD's overexpression suppresses the phenotypes [68]. These results suggest that *amx* may function at the level of the Notch S3 cleaves. More recently, another epistasis analysis was conducted between various forms of *Notch* and a mutant *amx*, encoding a derivative of *amx* with an N-terminus truncation (AMX^{ECD}), a potent inhibitor of Notch signaling [52]. This study revealed that overexpression of AMX^{ECD} suppresses the accelerated Notch signaling activity by NEXT or a ligand-dependent activated Notch, but does not suppress NICD [52]. Thus, taken

together, these results suggest that *amx* acts after the production of NEXT and before the production of NICD in the Notch signaling cascade.

Researchers have also started to understand the cell biological functions of *amx*. In embryos homozygous for *amx* (*amx* maternal/zygotic nulls), Notch is abnormally accumulated in the neuroectoderm even though such defective localization is restored with the progression of embryonic development [57] (Figure 4b). This observation suggests that *amx* may regulate the intracellular trafficking of Notch in a stage-specific manner [57] (Figure 4c). This idea is consistent with the results of epistasis analysis demonstrating that *amx* acts before the NICD production [52,68]. In addition to such functions of *amx* associated with the roles of the Notch signaling in embryonic development, studies have revealed the physiological roles of *amx* in adult flies [52]. The lifespan of adult flies homozygous for *amx* is shorter than that of wild types [52]. Additionally, an age-dependent decline in neural functions was observed in these adults [52]. Although it is

currently unknown whether these defects are associated with reduced Notch signaling, these results demonstrated that *amx* plays a crucial role in adult physiology and ageing.

The involvement of *amx* family genes in human diseases has also been reported. A rare missense variant (P155L) in *TM2D3* is significantly associated with an increased risk of developing late-onset Alzheimer's disease and an earlier age of onset [69]. This missense mutation behaves as a loss-of-function mutant of *TM2D3* in *Drosophila* [69]. Considering that *TM2D3* affects Notch signaling and Alzheimer's disease, the γ -secretase that plays crucial roles in both events may be a target of *TM2D3*. Accordingly, it has been proposed that *TM2D3* may participate in the intramembrane cleavage of Notch and APP by γ -secretase. Additionally, the P155L variant may influence the production of the β -amyloid peptide, which is similar to the pathogenic mutant forms of APP, Presenilin-1, and Presenilin-2 [69]. Thus, it was proposed that the Amx-family proteins are likely involved in γ -secretase functions

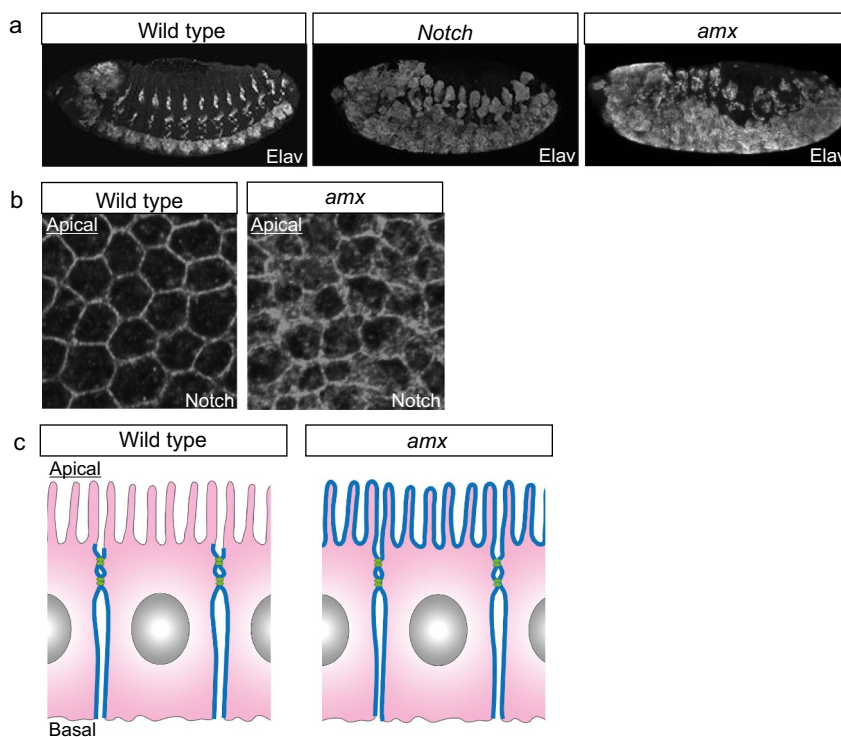


Figure 4. Amx Promotes Notch Signaling by Regulating the Notch's Subcellular Localization. (a) The lateral views of the wild-type (left, wild type), the *Notch* mutant (middle, *Notch*), and *amx* maternal/zygotic null embryos (right, *amx*) at stages 13–14. Neurons are visualized by anti-Elav antibody staining (white). (b) The distribution of Notch (white) at the apical regions of epithelial cells in the wild type (wild type) and *amx* maternal/zygotic null embryos at stage 5. (c) Schematics of the Notch (blue) localization in the epithelium of the wild type (left, wild type) and the *amx* maternal/zygotic null (right, *amx*).

and the production of the A β -42 peptide [67,68]. This idea is further supported by a previous finding that mammalian TM2D proteins bind the A β -42 peptide, which may help in the clearance of amyloid plaques by phagocytic glia cells [67]. Thus, the functional relevance between the *amx* family genes and the γ -secretase seems to be a promising subject for future research.

Maternal Notch Signaling in Other Animals

We will discuss the maternal effect of Notch signaling in various organisms. In invertebrate models, studies have revealed the essential roles of maternal genes encoding the components of the Notch signaling in embryogenesis. In *Caenorhabditis elegans* (*C. elegans*), there are two homologs of Notch, namely the *abnormal cell lineage-12* (*lin-12*) and *abnormal germ line proliferation-1* (*glp-1*) [70,71]. Mutants of *glp-1* were rediscovered based on their postembryonic sterility [72]. Subsequently, it was found that *glp-1* shows a recessive maternal effect of lethality because *glp-1* homozygous mothers produce only abnormal progenies regardless of the genotypes of embryos [73]. In the absence of the maternal *glp-1*, the AB-derived blastomeres, which normally produce pharyngeal cells, produce neurons instead [73]. In *C. elegans*, orthologs of *pcx* and *amx* have been reported [74,75]. However, phenotypes associated with their loss of functions have not. In sea urchins, a model invertebrate belonging to deuterostomia, *Notch* and *Delta* orthologs are expressed during early embryogenesis. Additionally, they play maternal roles [76]. Maternal genes of sea urchins maintain high expression until the two-cell stage, which decreases after the expression of zygotic genes [76]. Using a Morpholino, *Notch* and *fringe* proteins – which encode O-fucose-specific β -1,3 N-acetylglucosaminyltransferase – were knocked down at the two-cell stage [77]. These embryos fail to gastrulate, indicating that maternal Notch signaling requires glycosylation of Notch EGF-repeats by Fringe in sea urchins [77].

In contrast to invertebrates, the maternal effect's contribution to Notch signaling in vertebrates is still ambiguous. Mouse embryos that are homozygous for *O-fut1* and lack maternal contributions, in which maternal Notch signaling should be

abolished, normally developed until E8 [78]. In mice, the expression levels of maternal and zygotic genes are reversed at the two-cell stage (E1.5). Embryos lacking the maternal effect cannot survive the cleavage-stage corresponding to the first three cell divisions before forming a mature morula [79] (Figure 2). Therefore, Notch signaling is not essential for morphogenesis before gastrulation, indicating that the maternal effect is not involved in mammalian Notch signaling. However, the definition of maternal genes in mammals has been expanded. This is due to the discovery of new maternal genes that play an essential role in embryonic development after the two-cell stage [80]. Additionally, it has been reported that the administration of an inhibitor of Notch signaling to mouse embryos at the two-cell stage downregulates *TLE4*, an early naïve-pluripotency marker [81]. Therefore, further studies are required to determine whether maternal functions of genes play essential roles in Notch signaling.

Conclusions

Drosophila embryos complete cellularization and begin cell-cell interactions at stage 5. Their neuroblasts begin differentiation in the neuroectoderm at stage 8 [29]. Therefore, lateral inhibition through Notch signaling should occur during stages 5–8. Genetic screens based on the zygotic neurogenic phenotype occasionally failed to isolate maternal neurogenic genes. This is because many genes composing the Notch signaling pathway were found to have maternal contributions [42,46–52]. Most newly identified genes in Notch signaling are maternal neurogenic genes. Thus, further studies on maternal genes will be useful for identifying novel components of Notch signaling and other cell signaling pathways. This idea applies to organisms having large quantities of maternal mRNAs and proteins.

Most *Drosophila* maternal neurogenic mutants are also recessive zygotic lethal mutations. Thus, these maternal neurogenic genes also play zygotic roles that are essential for embryonic and postembryonic development. However, homozygotes of

pcx or *amx* family genes with their maternal contributions do not show postembryonic defects in development and are viable [50,66]. These phenomena can be explained if the maternal contributions of these genes persist until adulthood. However, alternatively, these genes may have specific roles only in the Notch signaling of the embryonic stage. In the latter case, we must consider another layer of complexity in Notch signaling regulation, such as stage-specific regulatory factors, which may further extend our understanding of this signaling pathway.

The MZT has been an important subject in developmental biology. Many maternal genes play essential roles in cell signaling. Accordingly, understanding the balance between the maternal and zygotic functions of genes involved in cell signaling and their regulation during the MZT is of the utmost importance.

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Data Availability

Data sharing does not apply to this article as no new data were created or analyzed in this study.

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No potential conflict of interest was reported by the author(s).

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