ARE γ-SECRETASE AND ITS ASSOCIATED ALZHEIMER’S DISEASE γ PROBLEMS?

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Abstract

Presenilins (PS1 and PS2) and the amyloid-β precursor protein (AβPP) are the only known proteins as causing monogenic Alzheimer’s disease. AβPP is not the unique substrate of the γ-secretase complex. Presenilins are also implicated in the processing of Notch, an important developmental protein, which is thought to compete directly with AβPP for cleavage by γ-secretase.

In the context of cleavages in alpha, beta and gamma and with the recent three-dimensional models of γ-secretase complex, a kinetic study of the sequential proteolysis of AβPP prompts us to think the possible existence of two entrance sites for substrate with only one exit site, a configuration depicting a lowercase gamma letter. The quantitative distribution of the cleavage products by the γ-secretase, mainly Aβ40, Aβ42 and Aβ43, could be explained in the context of this hypothesis.

Based on published results in the literature and the analyses of AβPP C99 fragment, highly abundant in Down’s syndrome patients, we propose that β- and γ-secretases can function as a supra-enzyme complex where AβPP substrate might be attached to the γ-secretase complex before β cleavage takes place. Different studies point that a small peptide sequence, showing homology in presenilins and AβPP, plays a pivotal role and that minor alterations in the sequence of AβPP protein limit the formation of C99 and also of Aβ40 and Aβ42.

The model proposed could be of importance in future studies aimed at understanding the specific events involved in course of AD pathophysiology and also at studying of formation/deterioration of memory.
Introduction

Only three genes have been identified as causing monogenic Alzheimer's disease: *PSEN1*, *PSEN2* and *APP* [1]. These genes encode presenilins (isoenzymes 1 and 2) and amyloid-β precursor protein (AβPP), respectively. The relationship between these proteins and the disease can be understood as an enzyme/substrate based association in which the enzyme is presenilin and AβPP is the substrate. However, presenilin is part of a larger complex called γ-secretase, which constitutes its catalytic core [2], and it cleaves more than just the AβPP molecule. The Notch receptor, a molecule involved in cell development and differentiation, is found within the list of molecules cleaved by presenilin [3-5].

The cleavage balance provided by γ-secretase over the more pathologically relevant substrates [6] of AβPP and Notch receptors favours AβPP when an excess of this molecule (or an excess of any of its products) interferes with cleavage of the second substrate (the Notch receptor) and *vice versa* [7].

Presenilin restricts the Notch receptor’s processing/signalling by substrate competition with a relative excess of the competitor AβPP substrate or an excess of its derived products. At the same time, presenilin modulates its own expression through negative feedback exerted by presenilin processing of the Notch receptor [8]. Consequently, presenilin becomes increasingly less accessible to the Notch receptor substrate after the Notch release. In vivo, the equivalent of interference by an excess of the AβPP substrate would be chromosome 21 trisomy [9], in which an overexpression of Notch1 and Delta-like protein 1 (Dll1, a Notch Delta ligand) in Down syndrome can be observed. This chromosome contains the gene locus of the AβPP protein.

In the proteolytic sequence of the AβPP molecule, α- or β-secretase is first cleaved. The α-secretase leads to the C83 fragment. Cleavage by β-secretase (BACE1) results in two potential fragments: C99 and C89. All of these fragments can undergo progressive sequence cleavage by γ-secretase at the transmembrane domain, starting at the beginning of the intracellular domain. This process releases the amyloid intracellular domain, AICD (mainly γCTF: 50-99 and 49-99), and, on the other side (to the N-terminal), the amyloid peptide Aβ (ranging from Aβ$_{40}$ to Aβ$_{37}$) is released, leaving three residues on every step of the cleavage sequence (Fig. 1) [10].

![Fig. 1: Classical APP processing sequence. Blue ring, β-cleavage site; white ring, α-cleavage site; red ring, γ-cleavage site.](image-url)
This process would result in two proteolytic cleavage sequences, based on the Aβ residue at which the sequence starts [10,11].

\[ \text{AICD50-99} + \text{Aβ}_{x-49} \rightarrow \text{Aβ}_{x-46} \rightarrow \text{Aβ}_{x-43} \rightarrow \text{Aβ}_{x-40} \rightarrow \text{Aβ}_{x-37} \]
\[ \text{AICD49-99} + \text{Aβ}_{x-48} \rightarrow \text{Aβ}_{x-45} \rightarrow \text{Aβ}_{x-42} \rightarrow \text{Aβ}_{x-38} \]

Recent discoveries about the structure of the γ-secretase complex have revealed the existence of two water pores or channels [12]. These cavities help to explain the hydrolysis of the peptide bonds within the hydrophobic environment of the membrane. Water channels in other membrane proteases (the serine protease GlpG or the metalloprotease Zn S2P [13]) are related to the entry of the substrate peptide, allowing the entry of the substrate into the catalytic site. In the case of the γ-secretase complex, an identical event could occur. If the water channels were, in fact, two distinct channels through which an amyloid type molecule, in addition to water, could access the active site of the complex while also suffering different strains and interactions with anchorage points in different manners, it would probably result in different products, as outlined in Ihara’s model of progressive proteolysis [11].

The two-channel γ-secretase hypothesis

In our two-channel γ-secretase hypothesis, a single active site (belonging to the monomeric γ-secretase complex) optionally receives the CTF β-cleaved substrate (C99) from two different sites or channels, and progressive enzymatic process occurs. The Aβ_{40} and Aβ_{42} products are the most abundant products of γ-secretase complex processing, and the two channels will lead to two cleavage sequences [10,11]. The γ40 channel will result in the Aβ_{40} product, and the alternative channel, γ42, will result in the Aβ_{42} product.

γ40: C99 \rightarrow \text{AICD50-99} + \text{Aβ}_{49} \rightarrow \text{Aβ}_{46} \rightarrow \text{Aβ}_{43} \rightarrow \text{Aβ}_{40} \rightarrow \text{Aβ}_{37}

γ42: C99 \rightarrow \text{AICD49-99} + \text{Aβ}_{48} \rightarrow \text{Aβ}_{45} \rightarrow \text{Aβ}_{42} \rightarrow \text{Aβ}_{38}

This model is supported by previous data from the Ihara group [14]. In that study, Kakuda et al. measured Aβ species that were generated by a solubilised γ-secretase assay system in which defined amounts of C99-FLAG were used. These researchers found that with increasing concentrations of C99-FLAG, the production of Aβ_{40} progressively accelerates and approaches a plateau over substrate concentration (i.e., a sigmoidal concentration-response curve), which is followed by a decrease that reveals an excess substrate inhibition. Although the production of Aβ_{42} also increases with increasing substrate concentration that is roughly in proportion to the increase in Aβ_{40}, its production does not decrease; therefore, Aβ_{42} does not seem to undergo substrate inhibition. Interestingly, the production of Aβ_{43} increases with increasing substrate concentration in a manner nearly parallel to that of Aβ_{42}, although a constant rate (V max) is not reached. At high substrate concentrations, the production of Aβ_{43} increases because of the substrate inhibition of Aβ_{40} production. All of these reactions converge at a point where the rates are similar.

We briefly outline a kinetic analysis. The enzyme system exhibits dual enzyme inhibition by the substrate, as shown in the Fig. 2.
Fig. 2. Substrate competition between the γ40 channel and the γ42 channel. Italic, the γ42 channel; Bold, substrate binding to the enzyme-substrate complex at the γ40 channel. S: Substrate; E: Enzyme; ES40: Enzyme-substrate coming from γ40 channel complex; ES42: Enzyme-substrate coming from γ42 channel complex; S42ES40: Enzyme-substrate complex resulting from integration of a new substrate coming from γ42 channel to ES40 complex.

Some inhibition should occur because a molecule of the substrate can enter two different sites (normal and italic scheme branches at Fig. 2) and this external inhibition between the channels is reflected by the apparent Michaelis constants ($K_m$) of each product, $K_{1m}$ and $K_{2m}$ (for $A\beta_{40}$, $K_{1m} = 407.91$ nM and the maximal $A\beta_{40}$ production rate: $V_{40\text{max}} = 205.38$ arbitrary units, AU [14]). Thereby we can analyse any of the two equilibriums (normal and italic scheme branches at Fig. 2) separately.

In contrast, enzyme inhibition at the γ40 channel (bold scheme branch, $K_{3m}$ Michaelis constant at Fig. 2), that occurs via binding between the substrate and the complex, starts at a given substrate concentration ($[S]$) near $[C99] = 1000$ nM (Fig. 3).

Three reactions are involved with the ES40 enzyme-substrate complex:

\[
E + S \rightarrow ES40, \quad k_1, \text{rate constant.}
\]

\[
ES40 \rightarrow E + S, \quad k_1, \text{rate constant.}
\]

\[
ES40 \rightarrow E + A\beta_{40}, \quad k_2, \text{rate constant.}
\]

$ES40 + S \rightarrow S42ES40$ reaction should not be included here, in order to simplify the analysis, because it may be the slowest reaction of the group as it depends on the concurrence of a new substrate molecule at the γ42 channel within the ES40 complex.

Let $S'$ be the effective amount of substrate that should access the ES40 enzyme-substrate complex through the γ42 channel. In consequence, it will depend on the ES40 enzyme-substrate complex amount and will be proportional ($k_s$, proportionality constant) to the missing amount of $A\beta_{40}$, $A\beta_{40\text{gap}}$ (the difference between the detected $A\beta_{40}$ amount and the $A\beta_{40}$ amount that would be detected on a Michaelis kinetics):

\[
[S'] = k_s \ast [A\beta_{40\text{gap}}]
\]

In addition, three reactions are involved with the S42ES40 enzyme-substrate complex:

\[
ES40 + S' \rightarrow S42ES40, \quad k_1', \text{rate constant.}
\]

\[
S42ES40 \rightarrow ES40 + S', \quad k_1', \text{rate constant.}
\]
$S42ES40 \rightarrow E + A\beta_{42} + A\beta_{43}, k_2$ rate constant.

At steady state:

\[ \frac{d[ES40]}{dt} = \frac{d[S42ES40]}{dt} = 0 \]

So, we have:

\[ k_{-1} *[ES40] + k_1 *[ES40] = k_{1}' *[E] \]

\[ k_{-1}' *[S42ES40] + k_2 *[S42ES40] = k_{1}' *[ES40] *[S'] \]

Rearranging, we obtain $K_{1m}$ and $K_{3m}$ Michaelis constants for the ES40 and S42ES40 enzyme-substrate complexes respectively, as follows:

\[ K_{1m} = \frac{(k_{1} + k_{1}')}{k_{1}} = \frac{[E]*[S]}{[ES40]} \]

\[ K_{3m} = \frac{(k_{1}' + k_{2})}{k_{1}} = \frac{[ES40]*[S']}{[S42ES40]} \]

We can solve for $[E]$ and $[S42ES40]$, respectively:

\[ [E] = \frac{K_{1m}*[ES40]}{[S]} \]

\[ [S42ES40] = \frac{[S']**[ES40]}{K_{3m}} \]

We analyze the A\beta\text{40} formation rate. Let $v_{40}$ be the A\beta\text{40} formation rate. This rate will be maximal when the whole amount of the enzyme ([E]\text{0}) is bound to the substrate, where ES40 is the enzyme-substrate complex.

\[ \frac{v_{40}}{V_{40\text{max}}} = \frac{[ES40]}{[E]_0} = \frac{[ES40]}{[E]_0 + [ES40] + [S42ES40]} \]

Substituting from Eq. #7, #8, #9 and #1, followed by simplification, result in the following Eq.:

\[ v_{40} = \frac{V_{40\text{max}}*[S]}{K_{1m} + [S]*\left(1 + \frac{[S']}{K_{3m}}\right)} = \frac{V_{40\text{max}}*[S]}{K_{1m} + [S]*\left(1 + \frac{k_s}{K_{3m}*[A\beta_{40\text{gap}}]}\right)} \]

From Eq. #10, we can estimate that $K_{3m}/k_s \approx 120$ (arbitrary units, AU). Fig. 3 shows how results fit the model, and yield an accumulated difference of 0.08 AU between experimental (after 3 h of incubation [14]) and estimated A\beta\text{40} amount (using Eq. #10).
If γ40 is preoccupied (which should occur more and more frequently with an increasing substrate concentration), then the substrate coming from the γ42 channel will interfere with the reaction at the γ40 channel in the previous step of the progressive proteolysis and will cause the previous product of the sequence to accumulate.

**The AβPP substrate might be attached to the γ-secretase complex before β cleavage takes place**

In the cortex of the PS1 cKO mouse, the release of α- and β-secretase CTF products was examined [15]. Two carboxy-terminal fragments, C89 and C83, clearly increased as a result of the increased availability of the AβPP substrate for the α- and β-secretase cleavage reactions, respectively. Both CTF products elevated by as much as 30-fold, whereas C99, the other β-secretase cleavage product, only increased threefold.

β-cleavage of AβPP, in vitro [16] and in vivo [17], preferably results in the production of C99 molecule. Zhou et al. [16] suggested that the relative abundance of BACE1 cleavage at the two adjacent sites is governed by the expression levels of the protease: when BACE1 levels are low, C99 cleavage product is the major specie, when BACE1 levels are high, C89 cleavage product becomes predominant. Apart from the increased availability of AβPP for β cleavage, the relatively small increase in C99 is explained in this context if a relative increase β- over γ-secretase is considered. Why is there such a small increase in C99 in the PS1 knockouts? It seems that, in this case, BACE1 C99 cleavage is linked to PS1, as BACE1 and PS1 interact directly [18]. Together, these observations suggest that AβPP might be physically attached to the γ-secretase complex before C99 β cleavage can occur.

In the two-channel γ-secretase hypothesis, a second substrate entering the channel leading to the catalytic site of the γ-secretase complex may be implicated (this would be the path of Aβ42 generation). This idea has become increasingly clear and is reinforced by Fabiana Renzi et al. [19]. Our interpretation of Renzi’s model is the 3D structure drawn in Fig. 4.
Fig. 4. The reconstructed 3D structure of the γ-secretase complex (showing the γ40 and γ42 channels).

Taking both the two-channel γ-secretase hypothesis and that AβPP substrate might be attached to the γ-secretase complex before β cleavage takes place, one arrives at the conclusion that β- and γ-secretases should be linked on the same plane (Fig. 5 left), as they are transmembrane enzymes that interact directly [18].

Fig. 5. The reconstructed 3D structure of the γ-secretase complex at the supracomplex. 5a) β- and γ-secretases linked on the same plane; 5b) showing the C-shaped APP_γ40 slice facing the extramembrane space, ready to be cleaved at the β position.

We suggest that the outer portion of a hypothetical AβPP substrate turns, creating a C-shaped slice in the extramembrane space, which is ready to be cleaved at the β position (Fig. 5b).

The radical insertion or deletion of residues between C99 and C89 β cleavage site has shown to lead to C89 product [20]. β-Secretase should be close to γ-secretase, and the distance between them should remain relatively constant (if any interaction exists). Thus, these spatial conditions should create a unique cleavage site, resulting in the C99 product. Progressive cleavage at the γ site (along the γ40 path) would subsequently lead to Aβ40.
What about the γ42 channel? The γ42 gate must be located opposite from the putative β-secretase position, as it is located opposite from the γ40 gate (Fig. 5a). An AβPP trying to enter this second gate should have previously been β-cleaved because it cannot access β-secretase (i.e., only a β carboxy-terminal fragment (β-CTF) should be γ-cleaved along this path). If the substrate is C99, it could only come from the γ40 path. However, how does C99 escape from the γ40 channel, and what is the driving force at the outset?

Evidently, some AβPP may enter the γ42 channel, but it will not be β-cleaved as previously described because there is no β-secretase available at this position (Fig. 5a). This second AβPP, coming from γ42 to reach the docking site, may interfere with the AβPP substrate docking at γ40. Consequently, it will make the AβPP molecule at γ40 leave this channel. When the substrate docking at γ40 is β-cleaved C99 it might leave γ40 without undergoing γ-secretase cleavage. This C99 fragment would be able to enter the γ42 channel, be γ-cleaved and even inhibit the reaction that occurs at the γ40 channel. Thus, C99 has a cascading or multiplicative effect on its own production and explains the tremendous increase in C99 levels that is observed in Down syndrome [17,21]

The question remains of how a molecule could interfere with the docking zone. The directed insertion of 3 leucine residues (N-LVMLLLLKKK-C; AβPP 3L) between the transitional L645 helix to the random L648 (N-LVML-C) zone and the intracellular domain of the AβPP molecule (N-KKK-C) [22] impairs the formation of homodimeric structures in the molecule. This change in the AβPP molecule produces a strikingly significant accumulation of C-terminal AβPP fragments (mainly α-CTF), which occurs in cells expressing AβPP 3L.

If the docking of AβPP onto the presenilin molecule within the γ-secretase complex was severely impaired, then the β-secretase C99 cleavage of AβPP would not occur and α-CTF formation would be significantly increased.

Taking into account all the previously mentioned, we can wonder whether it is possible that N-LVML-C is a portion of the AβPP docking segment, and the N-LVAVL-C sequence at C-terminal TMD6 (transmembrane domain 6) presenilin domain its γ-secretase counterpart which holds the AβPP molecule in place for the previously required β-secretase cleavage. We believe that there are several factors acting in favour of this idea.

Firstly, these two sequences (N-LVMLKKK-C and N-LVAVLCPK-C) show some degree of sequence homology. Second, if the substrate were a carboxy-truncated AβPP molecule lacking the AICD49-99 fragment (N-LVMLKKK…-C, which begins with the putative AβPP docking segment), it should almost never dock onto the presenilin, as both Aβ40 and Aβ42 are produced at very low rates with equal likelihood, probably because there is no substrate competition at the docking site [23]. In this case, the formation of a dimer-like bond related to the AβPP-presenilin docking site attachment should be impaired.

In addition, DAPT, a γ-secretase inhibitor, acts preferentially blocking γ-cleavage but still blocking ε-cleavage (AICD formation) [24], and might interact with the putative docking sequence of γ-secretase (N-LVAVL-C). Importantly, there was no AICD detected in the cells expressing AβPP 3L (as occurs by DAPT inhibition), and DAPT treatment had a quantitative effect on the accumulation of CTFs [22], suggesting a continually weakening effect of DAPT on the AβPP-presenilin docking site.

This relationship between AβPP and DAPT regarding the interference at the docking site is confirmed by the fact that if the γ-secretase substrate is the chimeric AβPP lacking the AICD49-99 fragment then it results in identical amounts of Aβ40 and Aβ42 and DAPT had
no inhibitory effect [23]. Thus, without effective AβPP docking onto the referred site on presenilin, cleavage is undercatalysed, and DAPT could not exert its inhibition over this docking site.

A corollary is that any amyloidal molecule that enters γ-secretase through the γ42 channel having a similar sequence to that of the AβPP (N-LVML-C) can interact with the docking site and releases the molecule (if any) occupying the γ40 channel.

**DISCUSSION**

To date, the possibility of two input channels for γ-secretase substrate has not been considered. If this is the case, each channel would determine the form of the enzyme interaction with the substrate, the physical strains of this interaction and the various relationships that need to be established as a result of the competition that arises between the channels for access to the substrates.

The physical existence of channels has been confirmed by the 3D images from each new publication on the structure of γ-secretase complex [19,25]. Only Osenkowski and Li [12] have proposed an explanation: the possibility that the two channels allow the passage of water into the active site. In the publication by Renzi et al. [19], one of the channels was shown to allow the substrate access the enzyme inside. In this paper, one channel and its spatial arrangement relative to the other channel can be estimated more clearly.

The arrival of the substrate inside the active site should be explained, but an explanation will probably require some degree of restructuring of the various transmembrane domains of presenilin to hold the substrate.

In our model of two input channels for the substrate, the catalysis of the γ40 channel is inhibited because of the arrival of the C99 substrate from the γ42 channel, favouring the alternative Aβ42 formation. Along with the aforementioned substrate inhibition during the production of Aβ40, the Aβ42 production relative to other substrate cleavages as result of γ-secretase inhibitors [26,27] and presenilin mutations seem to suggest an allosteric modulation. Although our two-channel hypothesis has not yet been demonstrated, it is currently the most satisfactory explanation because the potential binding site for an allosteric modulation has not yet been identified. A recent paper [28] refers to a binding site for allosteric modulation in the formation of Aβ40 versus Aβ42. This report refers to an allosteric modulation related to the competitive inhibition between both channels (normal and italic scheme branches at Fig. 2), which should change the apparent K_m of each enzymatic complex (K_{1m} and K_{2m}) and consequently the constant of the alternative inhibition process (K_{3m}, bold branch at Fig. 2).

The release of Aβ43 from the γ-secretase complex primarily would occur via the decomposition of the enzyme substrate complex (k43: EAβ43→E+Aβ43; Fig. 6) rather than the Aβ40 formation reaction (K43: EAβ43→EAβ40+3aa). However, with an increasing substrate concentration, substrate inhibition due to the formation of the trimer (K3m: EAβ43+C99→C99EAβ43) temporally overlaps with the aforementioned enzyme substrate complex decomposition reaction (k43: EAβ43→E + Aβ43). This results in the release of additional Aβ43 from the γ-secretase complex to the detriment of the Aβ40 formation reaction.
Fig. 6. The production of various amyloid products is the result of the balance of the reaction rates between the subsequent reaction in the progressive proteolysis (capital K) and decomposition of the enzyme-substrate complex with the subsequent release of the substrate (small k). As the rate of proteolysis slows (capital K, probably because of the progressive withdrawal of the substrate with respect to the active site), the decomposition of the enzyme-substrate reaction (small k) will occur more frequently than the proteolysis.

Lee et al. [29] have previously reported that the overexpression of BACE1 leads to a robust increase in the C99 product over the C89 product. However, there are significant caveats concerning this data. First, a mutant AβPP (AβPPswe) was used. Second, the high levels of BACE1 that were used would most likely give rise to a greater increase in the C89 product over the C99 product because high levels of BACE1 should break the β-γ-secretase stoichiometric ratio (1:1) if the previously described supra complex had formed.

Regarding the initial attachment of AβPP substrate to the γ-secretase complex, there is a report indicating that PS1 stabilises the C99 molecules independently of γ-secretase activity [30]. We have postulated here only about the initial of anchorage site of the amyloid molecules. Through a first and second cleavage (ε and γ, respectively), the entire site sequence (N-LVML-C) is lost. Therefore, it seems necessary that there should be other anchorage points or additional scaffold either along the transmembrane domain or beyond (i.e., at the extracellular domain of the AβPP molecule) to hold the substrate. Apparently, the remaining fragment is not gradually introduced into the cleavage site of the γ-secretase complex in this process. The active site appears to act on a molecule that remains longitudinally static in the bilayer. This process renders the subsequent amino acid cleavage less accessible because of the distance to the active site, and the sequential cleavage becomes progressively slow (Fig. 6). The concurrent destruction of the enzyme-substrate complex also becomes more and more competitive, resulting in the release of the amyloid substrate. This explains the quantitative distribution of the various products within each cleavage sequence [14].

Regarding the corollary, it seems that the processing of AβPP and Notch receptor substrates is regulated to maintain a set of molecules at certain concentrations and/or activity limits. For some of these molecules, there should be a compromise between the function that justifies its existence and the potential toxic effects produced by their existence. This was highlighted in an article by Bero et al [31], who reported that endogenous neuronal activity regulates the regional concentration of interstitial fluid Aβ, which drives local Aβ aggregation. The role of C99 needs further study, and it is possible that it can maintain a functional role in the previously described context, even while it manifests toxicity [32]. The same could be applicable to Aβ42. The toxicity of Aβ42 is known,
but this toxicity is only compatible with high Aβ_{42} values [33]. Otherwise, the intracellular messenger AICD49-99 (which is derived from the γ_{42} path) in the presence of the coupling protein Fe65 is transactivated to the cell nucleus, where it produces a significant increase in AβPP levels [34]. Owing to the ubiquitin-mediated proteolysis, the stability of intracellular messenger AICD49-99 (derived from γ_{42} pathway) is much lower than AICD50-99 [35] (derived from the alternative γ_{40} pathway). It seems that the mechanism of C99 processing by the γ_{42} pathway would result in increased but unstable intracellular AICD49-99 levels, leading to a decrease in the total levels of AICD. After a Notch trigger (that leads to an initial increase in the AβPP production [34]), this decrease in the AICD levels would help to keep the AβPP within a certain threshold level or state.

According to our model, an increase in AβPP production suggests that the γ_{42} path impairs the processing of any other molecule that must pass through the γ_{40} channel for cleavage. The Notch receptor can be found among these. This possibility agrees with the idea that Notch is a self-limiting trigger signal. AβPP and the secretases are states or registers because they tend to maintain specific concentrations or states for themselves. The Notch trigger produces changes in the tandem AβPP/secretases depending on the previous concentrations of these molecules at the cell membrane. This type of architecture exists at the level of computer memory, and an example is the flip-flop circuit or latch that is used to store state information [36]. A difference between the human and digital models is that in humans the Notch system contributes to memory formation [37].

It is anecdotal that γ-secretase could describe an enzyme complex whose input and output channels depict a lowercase gamma letter, γ.

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