Extensive research efforts have been conducted over the past decades to understand the processing of the Amyloid Precursor Protein (APP). APP cleavage leads to the production of the β-amyloid peptide (Aβ), which is the major constituent of the amyloid core of senile plaques found in the brains of patients with Alzheimer disease (AD). Aβ is produced by the sequential cleavage of APP by β- and γ-secretases. Cleavage of APP by γ-secretase also generates the APP Intracellular C-terminal Domain (AICD) peptide, which might be involved in regulation of gene transcription. Up to now, our understanding of the mechanisms controlling APP processing has been elusive. Recently, APP was found to form homo- or hetero-complexes with the APP-like proteins (APLPs), which belong to the same family and share some important structural properties with receptors having a single membrane spanning domain. Homodimerization of APP is driven by motifs present in the extracellular domain and possibly in the juxtamembrane and transmembrane (JM/TM) domains of the protein. These striking observations raise important questions about APP processing and function: How and where is APP dimerizing? What is the role of dimerization in APP processing and function? Can dimerization be targeted by small molecule therapeutics?

**How are APP Dimers Forming in the Cells?**

Amyloid Precursor Protein (APP) is a type I transmembrane protein expressed in many cell types including neurons. The neuronal isoform of APP is a 695 residue protein, with a large ectodomain and a short intracellular region. The structure of APP and of its different domains makes it look like a very polyvalent protein, but its exact function remains unclear. APP knock-out animals display only a mild phenotype including minor locomotor troubles, gliosis and long-term potentiation defects. Several possible functions of APP were described in models overexpressing APP or its metabolites, and might therefore poorly reflect the physiological function of the protein. However, the role of APP as neurotrophic factor, adhesion protein, and its implication in neurogenesis have been reported.

Despite our difficulty to assign a clear function to APP, its processing has been extensively studied and characterized. APP is processed by two catabolic pathways referred to as the non-amyloidogenic and the amyloidogenic pathways (Fig. 1A). The amyloidogenic processing of APP is initiated by β-cleavage within the lumenal/extracellular domain of the protein. The β-cleavage of APP is performed in the brain by BACE1, an integral membrane protein belonging to the aspartyl protease family. The cleavage at the β-site produces a 99 residue membrane-anchored APP C-terminal fragment (BCTF), which is further cleaved by γ-secretase to generate Aβ. The γ-secretase activity is exhibited by a high molecular weight multiprotein complex formed by at least four proteins: a Presenilin (PS1 or PS2), Nicastrin (Nct), Pen-2 and Aph-1. The activity of the γ-secretase complex is also required for...
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The N-terminal region of APP, referred to as the E1 region that contains a Growth Factor-Like Domain (GFLD) and a Copper-Binding Domain (CuBD). A loop formed in that region by disulfide bridges seems to be required for the surrounding residues to acquire the conformation necessary for the stabilization of the homodimeric state (Fig. 2).

A further look at the regions potentially involved in APP homodimerization has focused attention on the juxtamembrane (JM) and transmembrane (TM) sequences of the protein. APP contains three glycine-xxx-glycine (GxxxG) motifs at the extracellular JM/TM boundary (Fig. 1B). It is a typical feature of the APP TM domain since the other members of the APP family (APLPs) do not contain GxxxG motifs in their TM region. These GxxxG motifs were originally shown to mediate sequence-specific dimerization and very close apposition of TM helices of the glycophorin A (GpA) protein. In GpA, the sequence LIxxGVxxGVxxT mediates tight dimerization between TM helices by direct glycine-glycine contacts. It has later been recognized that GxxxG motifs, and to a lesser extent AxxxA motifs, can mediate more generic oligomerization of TM domains. More importantly, it has been established that glycine is compatible with α-helical secondary structure in lipid bilayers and that, due to its small size, this residue allows the close association of interacting helices. These observations are of particular interest since the GxxxG motifs in the APP TM domain may participate in dimerization and are located in the region where cleavage of the protein occurs. In addition, the APP TM domain also possesses a GxxxA motif, which is predicted to be located on another interface. This observation would suggest—a priori—that dimerization of APP, and particularly of C99 (βCTF), where such GxxxA motifs would be very relevant, could be mediated by two distinct interfaces, allowing different dimeric states that could differentially regulate the cleavages occurring in the same region. Structural studies on the APP JM/TM region in isolation showed that the GxxxG motifs involving Gly625, Gly629, and Gly633 (APP695 numbering) mediate TM helix homodimerization.

On the basis of its structural features, APP has been proposed to bind extracellular matrix proteins like heparin and collagen and to have a receptor-like function. APP has been shown to form homodimers in a way that is reminiscent of dimerization described for other transmembrane domain receptors, like the EGF receptor. For the full-length protein, dimerization is induced by the generation of the intracellular fragment named AICD (APP Intracellular C-terminal Domain). AICD was shown to translocate to the nucleus, and there is growing experimental evidence suggesting a role for AICD in the regulation of gene transcription, even if the identity of APP target genes remains a matter of debate. APP processing, as part of its function, is likely to be a critical event in the onset and progression of Alzheimer’s disease (AD), not only because it leads to Aβ production, but also because it controls the intracellular signaling associated with APP, which in turn might regulate the expression of genes involved in the disease. Unfortunately, the mechanisms regulating APP processing and their impact on APP function are so far poorly understood.

Figure 1. Schematic representation of APP processing. (A) In the non-amyloidogenic pathway, cleavage by the α-secretase generates soluble APP (sAPP) and a membrane-anchored C-terminal fragment (sCTF) that is further processed into a 3 kDa fragment (p3) and APP Intracellular C-terminal Domain (AICD). In the amyloidogenic pathway, cleavage by the β-secretase generates sβAPP and sCTF; the latter is cleaved by γ-secretase leading to β-amyloid (Aβ) peptide and AICD production. (B) Schematic picture of the juxtamembrane and transmembrane region of APP. The aminoacids of the Aβ sequence are in a red box. The positions of the cleavages by α-secretases (α), β-secretase (β), and γ-secretase (γ; positions corresponding to Aβ 40 and 42 and ε; corresponding to the N-terminus of AICD) are indicated by arrows. The aminoacids of the transmembrane domain are underlined. Glycines and alanines from the GxxxG or GxxxA motifs are in white. Numbering is according to the sequence of APP695.

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in lipid bilayers, and that the GxxxA motif is outside the dimeric interface.  

Mutational studies of APP indicate that these GxxxG glycines are important in vivo for C99/βCTF dimerization. However, their influence on dimerization has been a matter of debate. For example, mutation of Gly625 and Gly629 to isoleucine diminishes the ability of the APP TM sequence in isolation to dimerize in ToxCAT assays. This system (ToxCAT) allows the study of transmembrane helix-helix oligomerization in a natural membrane environment. It uses a chimeric construct composed the DNA binding domain of a dimerization-dependent transcriptional activator fused to the TM of interest. Dimerization the TM regions can be monitored by the activation of a reporter gene. It has been widely used to study dimerization of glycoporphin A (GpA) TM region. It is to note that these assays are carried out in bacterial models that might not well reflect the natural environment found in eukaryotic cell membranes. Studies in CHO (mammal) cells showed that the mutation of the Gly625 and Gly629 residues to leucine or isoleucine seems to allow dimerization of the entire C99 protein in cell lines, possibly via another interface, indicating that TM dimerization/oligomerization might be cell specific or dependent on the structure and the composition of the bilayer in which it is anchored. These results have been obtained mainly by expressing not the APP holoprotein, but its amyloidogenic fragment (C99) in cells, and the role of the GxxxG motifs in homodimerization of the full-length protein is rather speculative. It is likely that the GxxxG motifs play a marginal role in full length APP homodimerization, which is mainly controlled by the ectodomain. Further functional and structural studies on the entire C99/βCTF and not only on the TM domain would be interesting to establish the structure (helical or not) of extracellular JM region and identify the residues involved in the stabilization of the TM/JM dimers.

### The Role of Homodimerization in APP Processing and Function

Homodimerization could represent an important step in APP processing and thus play a regulatory role in APP function. Up to now, two processes can be taken into account for APP homodimerization, involving either the ectodomain or the TM region.

Structural studies showed that the E2 region from the ectodomain can reversibly dimerize in an antiparallel orientation. The existence of trans-cellular dimers, involving E1 regions of the extracellular region, has been reported in mouse brain and synaptic compartment, indicating that dimerization participates in cell adhesion and cell-cell interaction. This trans-dimerization can occur not only in APP-APP interactions, but also in heterocomplexes of APP family proteins containing either APP or APP-like proteins (APLPs). This intercellular dimerization process has been described for proteins that belong to the cell adhesion molecule (CAM) family, and it supports the role of APP in neuronal migration during embryogenesis.

Dimerization of the JM/TM domains of APP C-terminal fragments might have different consequences. As mentioned before, these domains are precisely the regions in which APP is cleaved by the secretases (α, β and γ). Introduction of a cysteine residue at the junction of the JM/TM region allows the formation of stable dimers linked by disulfide bridges. These mutants show increased Aβ production. Interestingly, Aβ is produced as a stable dimer, indicating that the amyloidogenic secretases (β and γ) are able to process APP under its dimeric form. This observation represents an interesting proof-of-concept supporting the hypothesis that Aβ production is triggered by the dimerization of the amyloidogenic stubs of APP (βCTF/C99). Nevertheless, one restriction to this model is that dimerization is forced by interchain disulfide bridges that do not naturally occur. The situation is far more complex in naturally occurring dimers of APP C-terminal fragments. In this context, the GxxxG motifs might allow the close apposition of the TM helices and the tight packing of dimers by hydrogen bonds formed by residues surrounding the GxxxG motifs. Mutagenesis of the GxxxG motifs has suggested two important ideas (see Fig. 3). First, the strength of the interhelical interactions controlled by the GxxxG motifs regulates processing by the γ-secretase. The critical residues are Gly625 and Gly629. Considering that the γ-secretase can process its substrate when it is dimeric, dimerization would modulate the accessibility of the enzyme to the cleavage site. It is important to consider in this model that dimerization is not affecting the accessibility to the different γ-cleavage sites in a similar manner. For instance, loss of dimerization via the GxxxG motifs has been shown to inhibit the production of Aβ42, the long neurotoxic isofrom. Importantly, cleavage by γ-secretase is also regulated by the precise orientation of the helices in the dimer (Fig. 3). In the case of mutations or in a particular membrane context, helices can rotate to form dimers through other

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**Figure 2.** The different domains of APP and its dimerization. The ectodomain of APP contains two major regions (E1 and E2). E1 contains a copper-binding domain (CuBD), separated by a loop (red bold line) from the Growth Factor-like Domain (GFLD). The E1 and E2 domains are linked by and acidic region (black bold line). The E2 region is essentially formed by the Central APP domain (CAPPD). The APP770 and 751, which are the isoforms expressed in peripheral tissues, contain a Kunitz Protease inhibitor domain (KPI) that is absent from the neuronal isofrom (APP695). JM/TM, juxtamembrane and trans-membrane regions. IC, intracellular region.
Further characterization, cleavage at dimerization has similar regulatory roles therefore of prime interest to understand if of A and AICD could play an essential role in the Notch protein. It was suggested, by www.landesbioscience.com does not occur at the γ-position cleaved by the ε-cleavage and AICD release could be maintained but cleavage at γ-positions and Aβ release strongly impaired.

The role of dimerization in the release of AICD has been addressed in the same studies. The cleavage producing AICD does not occur at the γ-positions used for Aβ production but at a site referred to as ε, which is located more closely to the TM/juxtamembrane region and corresponds to the position cleaved by the γ-secretase in the Notch protein. It was suggested, by analogy with Notch, that AICD could be involved in nuclear signaling and gene transcription. This suggestion gave rise to conflicting results and interpretations, but very recent work unraveled new genes regulated by AICD and showed that their regulation relies on an epigenetic mechanism. Although this mechanism awaits further characterization, cleavage at ε site and AICD could play an essential role in APP function or during AD, independently of Aβ, by controlling gene expression. It is therefore of prime interest to understand if dimerization has similar regulatory roles in Aβ and AICD production. In fact, the mutations of GxxxxG motifs that strongly inhibit Aβ production/release do not affect cellular AICD levels. This apparent discrepancy could be explained by structural studies on the TM domain and the cytosolic JM region that showed the TM helix is disrupted at the intracellular membrane boundary near the ε-cleavage site. The helix-to-coil transition is required for the ε-cleavage by γ-secretase activity. A comprehensive model for APP processing in its intramembrane region can therefore be proposed. The progressive cleavage mechanism for APP proteolysis depends on the helix-to-coil transition at the JM/TM boundary and unraveling of the TM α-helix. This cleavage is not sensitive to the association of the TM domains. APP TM domain is further processed to the ε-cleavage site. Its processing of APP at the ε-cleavage positions is regulated by AICD and showed that their production/release do not affect cellular AICD levels.

Finally, it is important to note that this conceptual frame involving GxxxxG motifs in APP processing can be extended to heterodimerization. The GxxxxG or AxxxA motifs are also present in the TM domains of the BACE1 and the APH-1 proteins. The GxxxxG motif of APH-1 is critical for the assembly and the activity of the γ-secretase complex. Together, these data lead to the interesting hypothesis that the GxxxxG motifs could also be involved in the docking of APP to the proteases or multiprotein complexes responsible for its amyloidogenic processing.

Therapeutical Outcomes

The analysis of APP dimerization provided new insights in the complex steps of its processing. An emerging picture underscores the importance of TM dimerization in Aβ release. Strikingly, the motifs involved in dimerization of APP C-terminal fragments are also responsible for the packing of Aβ peptides into protofibrillar structures. Thus, the glycines present in GxxxxG motifs allow not only protein-protein interaction in the α-helical context of TM helices, but are critical in the formation of the cross β-sheet structures found in the Aβ fibrils. Peptide inhibitors based on a GxxFxGxF framework were shown to disrupt sheet-to-sheet packing and inhibit the formation of mature toxic Aβ fibrils. This is of particular interest to design specific inhibitors of Aβ oligomerization.

In a more general perspective, one can consider that any type of molecule able to promote a proper dimerization of APP TM regions will contribute to its amyloidogenic processing. This will require a better understanding of the precise structure of the JM region, as well as the characterization of the residues critically involved in stabilization of the dimers. In computational studies, Ser623 mediated stable hydrogen bonding interactions between the JM/TM helices and thus may be a key residue in the dimers of APP C-terminal fragments. It is very interesting to note that Ser623 is a critical determinant for the amyloidogenic processing of APP at the γ position. Its mutation to leucine drastically reduces Aβ production. Selective lowering of Aβ levels with small molecule non-steroidal anti-inflammatory drugs appears to be a promising avenue for therapy. Such molecules block γ-secretase cleavage by targeting not the γ-secretase complex but the region encompassing residues 28–36 of Aβ. It is precisely this region that contains sequence determinants of dimerization/oligomerization, such as the GxxxxG motifs. Antibodies mapping to an epitope in this Aβ region are also able to...
significantly reduce the accumulation of intracellular Aβ,36 that is known to be highly neurotoxic.28 It becomes therefore crucial to determine whether the structure of this region changes before and after cleavage, and how dimerization influences this process. This emerging field should be further investigated to improve the efficiency of immunotherapies aimed at treating AD, and also to design efficient and specific small molecules able to modulate Aβ production and oligomerization.

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