Minireview

Fe65 and the protein network centered around the cytosolic domain of the Alzheimer's β-amyloid precursor protein

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Abstract A distinctive tract of all the forms of Alzheimer's disease is the extracellular deposition of a 40–42/43 amino acid-long peptide derived from the so-called β-amyloid precursor protein (APP). This is a membrane protein of unknown function, whose short cytosolic domain has been recently demonstrated to interact with several proteins. One of these proteins, named Fe65, has the characteristics of an adaptor protein; in fact, it possesses three protein-protein interaction domains: a WW domain and two PID/PTB domains. The interaction with APP requires the most C-terminal PID/PTB domain, whereas the WW domain is responsible for the interaction with various proteins, one of which was demonstrated to be the mammalian homolog of the *Drosophila* enabled protein (Mena), which in turn interacts with the cytoskeleton. The second PID/PTB domain of Fe65 binds to the CP2/LSF/LBP1 protein, which is an already known transcription factor. The other proteins interacting with the cytosolic domain of APP are the G_o heterotrimeric protein, APP-BP1 and X11. The latter interacts with APP through a PID/PTB domain and possesses two other protein-protein interaction domains. The small size of the APP cytodomain and the overlapping of its regions involved in the binding of Fe65 and X11 suggest the existence of competitive mechanisms regulating the binding of the various ligands to this cytosolic domain. In this short review the possible functional roles of this complex protein network and its involvement in the generation of Alzheimer's phenotype are discussed.

Key words: Fe65; β-Amyloid precursor protein; Protein-protein interaction; Alzheimer; Amyloid

1. Introduction

The molecular basis of Alzheimer's disease (AD) is not fully understood and many apparently unrelated proteins are probably involved in the generation of the cellular and molecular phenotypes of the disease. The distinctive tract of AD at cellular level is represented by the degeneration of specific neuronal populations in the central nervous system. Histological analysis of affected brains drew the attention of research to several characteristics of the disease, that include the presence in the brain of amyloid plaques, neurofibrillary tangles and vascular alterations. Amyloid plaques mostly consist of a deposit of amyloid fibers, whose main constituent is a 40–42 amino acid-long peptide, named β-amyloid peptide (βA). One possible hypothesis to explain AD pathogenesis is centered on the neurotoxic effect of βA and/or of amyloid fibers [1,2]. Despite the huge amount of experimental results available, this hypothesis was not fully demonstrated [3].

An important contribution to the understanding of AD pathogenesis came from the identification of the genes whose mutations are linked with the familial forms of AD (FAD). Considering that the FAD phenotype is similar to that of AD, but for the early onset of the disease, it is conceivable that the functions of the proteins encoded by these three genes could be altered, and thus are responsible for the sporadic forms of AD as well. These three genes encode the β-amyloid precursor protein (APP) [4], presenilin 1 and presenilin 2 (PS1, PS2) [5]. Furthermore, the ε4 allele of the apolipoprotein E gene is associated with an increased risk of late onset AD [6].

The APP gene encodes several different isoforms of APP as a consequence of alternative splicing events; all these forms are membrane proteins with a single transmembrane tract, a large extracellular/intraluminal (E/IL) domain and a small cytosolic (CY) domain. APP undergoes complex alternative proteolytic processing events, giving rise, in the case of the α-secretase pathway, to the soluble form of the entire E/IL domain and to a C-terminal fragment [7] or, in the case of the combined action of the β- and γ-secretase pathways to a shorter soluble form of the E/IL domain, to the βA peptide and to a shorter C-terminal fragment [8,9]. Again, the functional significance of these proteolytic pathways is still unknown, but it is well demonstrated that they take place in different intracellular compartments: the α-secretase pathway, which prevents the formation of the βA peptide and thus is not amyloidogenic, takes place near or at the plasma membrane [10], whereas the β-secretase pathway (amyloidogenic) takes place at endosomal/lysosomal structures [11] and at Golgi-derived vesicles [12] and/or in the ER/intermediate compartment [13].

The functions of APP and presenilins are poorly (or not) understood and it is evident that defining these functions would be greatly relevant for the understanding of the molecular bases of AD. While no ligand has been clearly demonstrated for the large EC/IL domain of APP, the analysis of its short CY domain opened a stimulating new research area. In fact, starting from the observation that the Fe65 protein interacts with the APP cytodomain [14], many research data have documented the existence of a protein-protein interaction network centered to the APP cytodomain.

2. Structure and expression of Fe65, a new adaptor protein

Fe65 was originally identified as an EST corresponding to an mRNA expressed at high levels in the rat brain. The se-

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quence of the full length Fe65 cDNA revealed that it encodes a protein of 711 amino acids and a first comparison of this amino acid sequence with those contained in data banks revealed that a region of Fe65 can be aligned with a region of the retroviral integrases, DNA binding proteins which catalyse the integration of the proviral DNA into the host genome [15]. This observation, together with the demonstration that a short segment of Fe65, flanking at the N-terminal side the integrase homology, is a strong transcription activator, suggested the hypothesis that Fe65 could function as a transcription factor [15]. Following the discovery of two novel protein-protein interaction domains, the PID/PTB domain [16] and the WW domain [17], it was suggested that Fe65 has the characteristics of an adaptor protein, possessing a WW domain and two distinct PID/PTB domains. As summarized in Fig. 1, the WW domain of Fe65 covers the residues from 254 to 290, whereas the two PID/PTB domains, referred to as PID1 and PID2, are located from residue 365 to 510 and from residue 538 to 665, respectively.

The WW domain was originally discovered in the protooncogene-Yes-associated protein (YAP) [17], and it mediates the interaction with proteins containing XPPXY or PPLP consensus motifs [18]. More than 40 proteins have been identified which possess one or more WW domains and at least two types of this motif exist. The Fe65 WW belongs to the type II group, and it is expected that the similarity among various WW-containing proteins implies a similarity in the functional characteristics.

While the WW domain of Fe65 is conserved compared to those found in other proteins, the Fe65 PID/PTB domains significantly diverge from those found in Shc, IRS1 and other proteins. The alignment of PID1 and PID2 with the PID/PTB domain of Shc [19] and the data concerning the tridimensional structure of Shc [20] demonstrated that: (i) the Fe65 PID/PTB domains are shorter than that of Shc, so that the long $\alpha 2$ helix of Shc is absent from both PID1 and PID2; (ii) three key basic residues of Shc, involved in the interaction with the phosphate moiety of the NPXpY element, cannot be aligned with any similar residue in the Fe65 domains; (iii) the most conserved region among the three domains is the C-terminal part, including the $\alpha 3$ helix of Shc; however, the Phe^{198} residue of Shc, contained in this region, whose mutation completely impairs the binding activity of Shc [21], is substituted in both the Fe65 PID/PTB motifs by a cysteine. In agreement with these last observations, chimeric proteins, in which the C-terminal α helix of Shc is substituted with the C-terminal regions of both the Fe65 PIDs, are unable to interact with tyrosine phosphorylated receptors [19].

Northern blot analyses demonstrated that the rat Fe65 mRNA is detectable only in the brain [15], and only RNase protection experiments allowed the detection of low levels of this mRNA in other tissues. The mouse Fe65 mRNA is also detectable in other tissues, although these levels are at least two orders of magnitude lower than in the brain [22]. The mapping of Fe65 gene expression in the various tissues demonstrates that during the development Fe65 mRNA is restricted, starting from day E10, to specific areas of the nervous system (NS) of the mouse embryo and it is undetectable in other tissues [23]. The highest levels of this mRNA are present in all the ganglial structures of the NS and of the sense organs and in the cortex, particularly at the level of the hippocampus [23].

In cultured cells the in vivo observed differences between neural and non-neural tissues are less evident. In PC12 cells the Fe65 mRNA and protein levels are significantly higher than in other cell lines, but in all the cell types examined the amount of Fe65 is higher than that observed in the corresponding tissues (unpublished results). This discrepancy between cultured cells and tissues is also evident from the study of the rat Fe65 gene promoter. In fact, the most proximal of at least two start sites of the promoter is strong in neural derived cell lines, but it is still active in non-neural cells [24], and the transcription factors that bind to and regulate this promoter are not neural-specific [25]. This probably means that Fe65 is not restricted to specific areas of the nervous system, but the levels of its expression are significantly higher in these districts compared to other tissues. Furthermore, the increase of Fe65 expression levels in cultured cells compared to the cognate tissues suggests the hypothesis that in vitro growth conditions require higher amounts of Fe65.

3. Interaction of Fe65 with APP and APP-related proteins

By using as a bait the Fe65 region containing the two PID/PTB domains, the screening of a brain cDNA library in the yeast two hybrid system allowed to isolate cDNA fragments encoding the cytodomain of APP and of an APP-related protein, APLP1 [14]. Based on the opposite approach, i.e. by

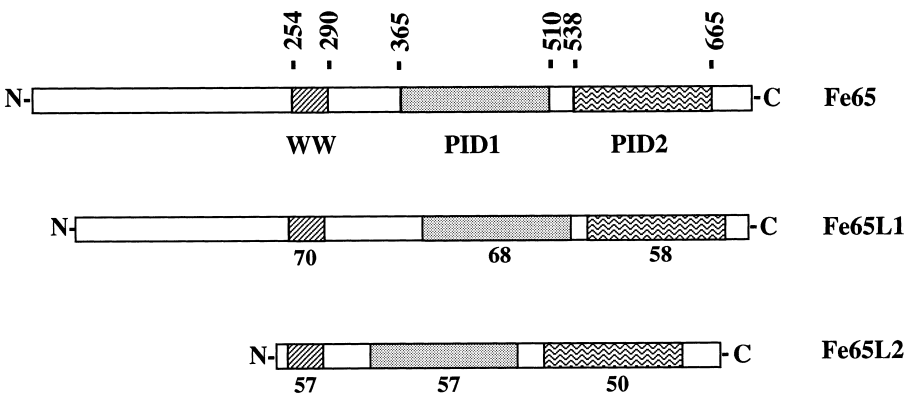

Fig. 1. Schematic representation of the domains of Fe65. The boundary residues of the three domains are reported in the upper part of the figure. In the lower part the representation of the other two members of the Fe65 protein family is reported, with the percentage of similarity of the domains compared to Fe65. The sequences used for the comparison are derived from the translation of: (i) for Fe65, the rat cDNA (AC: X60468); (ii) for Fe65L1, the human cDNA (AC: U62325); (iii) for Fe65L2, the rat cDNA (AC: Y13413).

using the cytodomain of APP as a bait, several research groups isolated cDNA fragments encoding Fe65 [22,26–28]. As demonstrated by in vitro and in vivo experiments APP and APP related proteins, APLP1 and APLP2, bind to the PID2 element of Fe65, whereas the PID1 element is completely dispensable [19].

Fe65 is an extremely conserved protein: its sequences in man, mouse and rat show more than 95% identity. It belongs to a multigene family. In rat and human there are at least three genes of this family, whose products have been named Fe65, Fe65L1 and Fe65L2 [26,29]. Fig. 1 shows a schematic representation of the structure of these three proteins, in which the three protein-protein interaction domains are significanly conserved, whereas most of the remaining parts of the proteins are unrelated. They are functionally very similar; they in fact interact with APP through the PID2 element, but also with the other members of the APP protein family. Rat Fe65 and Fe65L2 interact with high affinity with APLP2 and APP and to a lesser extent also with APLP1 [29]. Human Fe65L1 strongly interacts with APP and APLP2, but not with APLP1 [26]. The most significant difference among these proteins seems to be their tissue distribution: Fe65 mRNA is mostly expressed in the brain, and particularly in neurons of specific areas of the nervous system, whereas human Fe65L1 mRNA is ubiquitously expressed [26] and rat Fe65L2 mRNA significantly accumulates in the brain and in the testis [29].

The analysis of the interaction between Fe65 and the mutant forms of APP isolated from FAD patients demonstrated that the amount of Fe65-APP/FAD complexes found in cultured cells is significantly lower than those of Fe65 with wild-type APP. Particularly in CHO cells stably expressing the so-called Swedish mutant of APP the levels of the complexes between Fe65 and this mutant protein are undetectable [19].

4. The APP cytodomain is able to interact with various proteins

The APP cytodomain is able to interact with at least three other proteins, completely unrelated to Fe65. The first type of proteins that was identified as possible ligand of the APP cytodomain is represented by G_o protein. In fact, there are some results indicating that the peptide from residue 657 to residue 676 of APP binds to and activates the G_o protein [30,31]; this interaction mimics those of transmembrane receptors with trimeric G proteins; in fact, although the natural signal that uses the APP-G_o transduction machinery is not known, an anti-APP antibody is able to activate G_o [32]. The authors also observed that: (i) FAD-mutant APPs constitutively activate G_o [33,34]; (ii) FAD mutant APPs induce apoptosis in COS cells; and (iii) this phenomenon is mediated by the Gβγ subunit of G_o [35,36]. If confirmed, these results could be of great relevance to the AD phenotype.

A second protein interacting with the APP cytodomain is X11 [27]. This is a neuron abundant protein [37], which shares with Fe65 the characteristic of being a candidate adaptor protein; in fact, it interacts with APP through a PID/PTB domain, similar to those found in Fe65, and also possesses two other protein-protein interaction domains, belonging to the group of PDZ (PSD-95/Dlg/ZO-1) domains. Furthermore, as for Fe65, a second X11 protein (X11β) was identified. Very interestingly, it was recently demonstrated that transient overexpression of X11 in cultured cells provokes a decreased secretion of β-amyloid peptide in the medium [38].

Another possible ligand of the APP cytodomain was named APP-BP1 [39]. Very little information is available on this protein; its primary structure deduced from the cDNA sequence indicates that it is similar to the auxin resistance gene product AXR1 of *Arabidopsis* and to a protein in *Caenorhabditis elegans* of unknown function.

5. Possible competition among various proteins for the binding to the cytodomain of APP

The above described results demonstrated that at least four types of proteins can interact with the APP cytodomain. There are several experimental results suggesting that the contemporary binding of Fe65 and X11 seems to be impossible and that of Fe65 and G_o is not probable. Although no result is available concerning the region of APP involved in the interaction with APP-BP1, given the small size of the APP cytodomain, the competition between APP-BP1 and the other ligands of the APP cytodomain is conceivable.

Fig. 2 summarizes the experimental results obtained by using synthetic peptides or mutant forms of APP, indicating that the shortest APP peptide, which is still able to interact with Fe65, is represented by the C-terminal 32 residues [19]. This region includes the peptide interacting with X11 and significantly overlaps with the putative domain of binding to APP of G_o proteins. Furthermore, at least Tyr^{652} is not dispensable for the binding of both Fe65 and X11 [27].

The solution conformation of the APP cytodomain, studied by NMR and CD spectroscopy, indicated that the most prominent features of this peptide are two type I reverse turns adopted by the sequences TPEE and NPTY [40]. The NPXpY motif has been identified as the recognition site for the PTB/

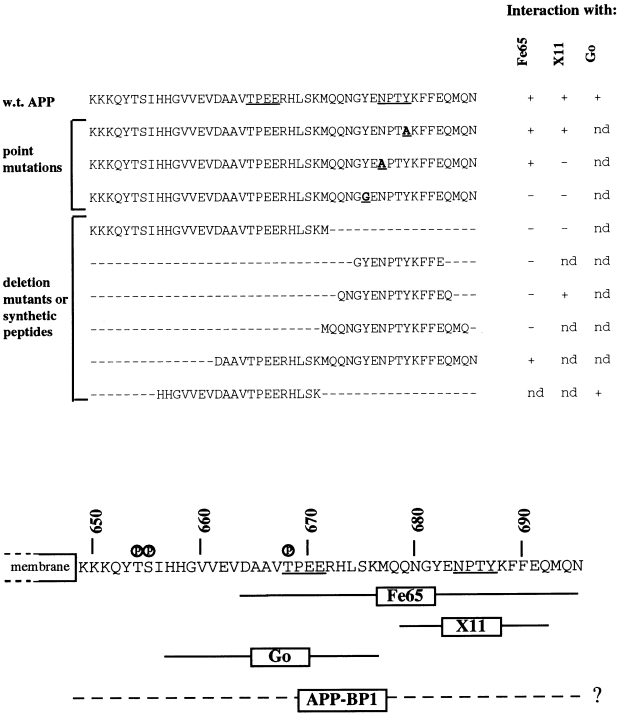

Fig. 2. Analysis of the APP CY domain interaction with its intracellular ligands. The type I turn motifs are underlined. The minimal regions of APP involved in the interaction with Fe65, X11, G_o and APP-BP1 are reported in the lower part of the figure. For the original results see refs. [19,27,30,40].

PID domains of Shc and IRS-1, whose binding to the EGFR or to the insulin receptor is strictly dependent on the phosphorylation of the tyrosine residue of the NPXY motif [41] and on the presence of hydrophobic residues in position −5 or −6 to −8 with respect to the phosphorylated tyrosine [42]. Comparing these data with those obtained with Fe65 and X11, it is evident that the PID/PTB domains of these two proteins have a significantly different peptide specificity compared to Shc and IRS1. In fact, although the NPXY motif is present in the APP cytodomain, the Fe65- and X11-APP interactions are independent from the tyrosine phosphorylation, and in addition the tyrosine of the NPTY motif can be changed into alanine without affecting the binding.

The other turn, TPEE, is included in the putative recognition sites of Fe65 and G_o, but a detailed analysis of this motif is necessary to evaluate its role for the binding of the two proteins.

The regulation of the binding of all these proteins to APP could be based on several mechanisms: (i) phosphorylation of the various partners; (ii) their different intracellular localization; (iii) regulation of their amounts; (iv) conformational changes preventing (or favoring) the binding.

Phosphorylation of APP was studied in detail: it is phosphorylated in vitro at three sites in the cytodomain by protein kinase C, calcium/calmodulin-dependent kinase and by cdc2 kinase [43], and in vivo by cdc2 kinase and other unidentified kinases [43,44]. The possible effect of these phosphorylation events on the binding of ligands to the APP cytodomain was not studied; however, Thr^{668}, which is phosphorylated by cdc2 kinase, both in vitro and in vivo, belongs to the above mentioned turn TPEE, which could be involved in the interaction with either Fe65 or G_o. Very little is known about the possible phosphorylation of the other proteins involved in the network. We recently demonstrated that Fe65 is phosphorylated [45]; this phosphorylation takes place in a region of the protein that does not seem to be involved in the interaction with APP.

Similarly to phosphorylation, the intracellular localization of APP was analyzed in great detail. Summarizing a huge amount of results, APP was found at plasma membrane, in the endosomal-lysosomal compartment and in the Golgi network. Therefore it is evident that this protein undergoes complex intracellular trafficking, which could have an important role in the targeting of APP to specific processing machineries. The NPTY motif present in the cytosolic domain of APP is identical to the signals responsible for clathrin-coated pit internalization, and in fact cell-surface APP is targeted to the lysosomal compartment [46]. A significant fraction of APP has been detected in neuronal cells in vesicular elements spread in all the cell and on the surface of dendrites and axons [47,48]. The axonal sorting of APP is independent from possible signals present in its cytoplasmic domain [49], whereas sorting of APP in MDCK cells to the basolateral surface is directed by a signal present in the cytodomain and including Tyr^{653} [50]. These results exclude the possibility that either Fe65 or X11 could have any role in this pathway, because mutation of Tyr^{653} has no effect on the binding of Fe65 and X11 [27] and because the deletion of the C-terminal 32 residues of APP, that contain the binding site of these two proteins, does not affect significantly the proper sorting of APP [50]. Therefore, it must be hypothesized that (an)other protein(s) interact(s) with APP at the region including Tyr^{653} and is responsible for the regulation of its basolateral sorting in MDCK cells.

6. Interaction of Fe65 with other proteins

Fe65 possesses two other protein-protein interaction domains, distinct from that interacting with APP: a WW domain and a second PID/PTB domain (PID1).

At least five putative polypeptides from mouse brain lysates have been purified by affinity chromatography with a GST-Fe65 fusion protein containing the WW domain; two of them have been identified by co-immunoprecipitation experiments as two isoforms of Mena, the mammalian homolog of the *Drosophila* enabled protein [51].

Mena cDNA has been isolated by the screening of mouse libraries using the *Drosophila* Enabled (Ena) as a probe [52]; it belongs to the Ena/VASP family of proteins; the main feature of this family, comprising four members, Ena, Mena, Evl and VASP, concerns the presence of two conserved N- and C-terminal Ena/VASP homology domains (EVH1 and EVH2, respectively) spaced by a central proline-rich region. Mena and VASP share several features: they are strongly enriched in focal contacts and in the cellular sites where active actin remodelling occurs, such as lamellipodia; both proteins are phosphorylated and a neuron-specific isoform of Mena, derived from the presence of an alternatively spliced exon, is phosphorylated at tyrosine residue(s) [52]. Mena, as well as VASP, is involved in many protein-protein interactions; its N-terminal EVH1 domain interacts with FPPPP regions present in vinculin, zyxin and the ActA protein from the intracellular pathogen *Listeria monocytogenes*; furthermore, Mena binds to profilin, a protein interacting with G-actin, through the polyproline region [52]. The Mena-Fe65 complex involves PPLP sequences present in the central, proline-rich region of Mena, as determined by filter binding and competition assays [51]. In order to elucidate the functional relevance of Mena-Fe65 interaction, it is very important to evaluate the potential involvement of such proteins in multiprotein complexes; if this is the case, Fe65 could act as an adapter protein transducing so far undefined stimuli involving APP to the cytoskeleton dynamics through Mena and downstream regulators of the actin polymerization, such as profilin. Furthermore, it has been shown that Mena strongly interacts with zyxin, which in turn is tightly related to focal contacts [52]. These observations are in agreement with the co-localization of APP in primary neurons with adhesion plaque components, that was suggested to support a role for the APP extracellular domain in mediating cell-matrix interaction [53].

The presence of Mena in the growth cones of P19 cells induced to differentiate to neurons by retinoic acid and the existence of a tyrosine-phosphorylated, neuron-specific isoform of Mena starting to appear, in mouse development, in the period of active neurite outgrowth, is suggestive of a potential involvement of Mena in neurite development; furthermore, ectopic expression of the neural isoform of Mena in fibroblasts induces the formation of F-actin-rich outgrowths resembling the microfilament-based extension of filopodia occurring during migration of the axonal growth cone [52]. The support for a possible relevance of the APP-Fe65-Mena system in the axonal development comes from the analysis of the neuronal phenotype of mice homozygous for an APP-null mutation, whose hippocampal neurons are less viable in pri-

mary cultures, present reduced ability to develop neurites and show reduced branching compared to wild-type neurons [54]; such phenotypes are in agreement with the suggested function of Mena in the axonal development.

Given the expected localization of APP-Fe65 and Mena-Fe65 complexes it was surprising to find that the ligand of the third protein-protein interaction domain of Fe65, the PID1 element, is an already known transcription factor, named CP2/LSF/LBP-1 [45]. Biochemical and cell fractionation analyses have demonstrated that specific isoforms of CP2/LSF/LBP1 are present, as expected, in the nuclear fraction, but also in the cytosolic membrane fraction [45]. The shorter CP2/LSF/LBP1 isoform (LSF-ID), probably derived from an alternative splicing and missing a 50-amino acid region encoding part of the oligomerization/DNA binding domain, is enriched in the cytosolic membrane fraction, while the full-length protein is mostly nuclear. On the other hand, even though most of the cellular Fe65 is associated with the cytosolic fraction, a discrete amount of the slowest-migrating, phosphorylated Fe65 isoforms have been found in nuclear fraction. In any case, the complexes between Fe65 and all the forms of CP2/LSF/LBP1 have been found in both nuclear and cytosolic membrane fractions [45].

The observed distribution of the two proteins and of the cognate complexes supports the hypothesis that Fe65 is involved in a regulatory mechanism underlying the targeting of the transcription factor to the nucleus. Among the possible cellular targets of CP2/LSF/LBP1, two gene promoters that are activated in early phases following mitogenic stimuli, the c-fos and the ODC promoters, respectively, possess putative DNA binding sites for CP2/LSF/LBP1. Interestingly, the DNA binding activity of CP2/LSF/LBP1 is also regulated by its phosphorylation in the earliest phases following mitogenic stimuli [55]; furthermore, preliminary data indicate that Fe65 itself is actively phosphorylated early following serum stimulation of serum-starved cells (unpublished results). Considering that the phosphorylated forms of Fe65 seem to accumulate preferentially in the nuclear fraction, it is likely that the phosphorylation of CP2/LSF/LBP1 and Fe65 in the early phases of the cell cycle regulates the translocation of Fe65-CP2/LSF/LBP1 complexes to the nuclear fraction. Another possibility to consider concerns the direct involvement of Fe65 in the formation of DNA-protein complexes with CP2/LSF/LBP1; since the CP2/LSF/LBP1 protein region involved in the interaction with Fe65 is located downstream the dimerization/DNA binding domain, the possibility of interacting with the DNA by a CP2/LSF/LBP1-Fe65 complex cannot be excluded. Interaction with Fe65, instead, involves the C-terminal region of the protein, in which the putative, glutamine-rich transactivation domain of CP2/LSF/LBP1 is contained [56]. In this case, Fe65 could affect the transactivating properties of CP2/LSF/LBP1 by masking its transactivation domain. Moreover, the possibility that Fe65 itself could activate the transcription cannot be excluded. In fact, it has been shown that a region of Fe65 possesses intrinsic transactivating properties when fused to the yeast GAL4 DNA binding domain, being able to transactivate a CAT reporter gene controlled by GAL4 *cis*-elements in co-transfection experiments [15].

The distribution of Fe65 between the nuclear compartment and the other districts of the cell must be studied in more detail. The results available at this moment indicate that the targeting of Fe65 to the nuclear fraction is regulated, and a phosphorylated region flanking the N-terminal side of the WW domain is necessary for this regulation [45]. It is not yet clear whether Fe65 is located within the nucleus or at perinuclear membranes; this last possibility deserves particular attention. In fact, a localization of PS1 and PS2 at the nuclear membrane in association with kinetochores and centrosomes was recently demonstrated [57]. This interesting observation supports the hypothesis that Alzheimer's mutant presenilins could generate chromosomal missegregation and/or could alter gene expression giving rise to the AD phenotype [57]. Given the existence of APP-PS complexes in living cells [58,59], it seems conceivable to postulate that Fe65 could be associated, together with APP and PS, to the inner nuclear membrane.

7. Perspectives

Fig. 3 summarizes the scenario that can be outlined around the APP cytodomain. At least three molecular machineries have been suggested to compete for this small domain. First, it can be hypothesized that a G_o protein-centered machinery transduces unknown extracellular or intraluminal signals to the cytoplasm and to the nucleus; several results suggest that this machinery controls cell apoptosis and could be involved in the pathological phenotypes associated with FAD mutations. Second, X11 protein has the characteristics of an adaptor protein, whose binding to APP is expected to be alternative to that of Fe65. It could link APP to other proteins by its PDZ domains. The ligands of these domains are not known, but it could be hypothesized that they are involved in the regulation of APP processing. Lastly, Fe65 seems to be the structural element connecting APP to the cytoskeleton through Mena, which in turn interacts with profilin-actin complexes or to zyxin or to vinculin. This connection could represent the molecular basis of the suggested role for APP to regulate axonal targeting through cell-matrix and cell-cell interactions. On the other hand, Fe65-CP2/LSF/LBP1 complexes suggested that the APP network is also able to affect gene regulation; this could play an important role in the neuronal molecular differentiation related to synaptic plasticity and involved in the learning process. This complex scenario probably has further degrees of complexity, given the finding that another protein, APP-BP1, is able to interact with

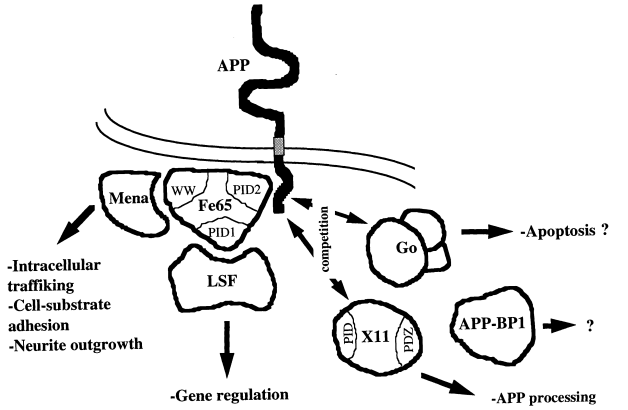

Fig. 3. Schematic representation of the protein-protein interaction network centered to the APP cytodomain. Some possible downstream targets of the network are indicated.

APP, and considering that the existence cannot be excluded of other not yet identified ligands of the juxta-membrane segment of the APP cytodomain, which could be involved in the demonstrated baso-lateral sorting of APP in MDCK cells. The finding that APP and PS interact could help to find a coherent model to explain how the dysfunction of two apparently unrelated proteins generates a single pathological phenotype. The CY domain of APP does not seem to be involved in the interaction with PS1 and PS2 [58]; therefore, the described protein-protein network is not the bridge between APP and PS. However, an indirect connection between presenilins and the various components of the network must be taken into account.

Future directions of the research have to be aimed at the elucidation of the possible role of the described protein-protein interaction network in: (i) APP processing and βA generation; (ii) APP and presenilins trafficking among the various intracellular compartments; (iii) APP and presenilin involvement in apoptotic process; (iv) regulation of gene expression. In any of these fields the functions of the APP-centered protein network could contribute to fill the numerous gaps in the knowledge of AD pathogenesis and also, more importantly, could become a target for the design of therapeutic agents to be developed in the next future.

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