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An X11α/FSBP complex represses transcription of the GSK3β gene promoter

Kwok-Fai Lau[1], **Michael S. Perkinton**[2], **Lilia Rodriguez**[2], **Declan M. McLoughlin**[3], and **Christopher C.J. Miller**[2]

[1] Department of Biochemistry (Science), The Chinese University of Hong Kong, Shatin NT, Hong Kong SAR. [2] MRC Centre for Neurodegeneration Research, King's College London, Institute of Psychiatry, London SE5 8AF, UK [3] Department of Psychiatry and Trinity College Institute of Neuroscience, Trinity College Dublin, St Patrick's Hospital, Dublin 8, Ireland.

Abstract

X11α is a neuronal adaptor protein that interacts with the amyloid precursor protein (APP) via a centrally located phosphotyrosine binding (PTB) domain to inhibit production of Aβ peptide that is deposited in Alzheimer's disease brains. X11α also contains two C-terminal postsynaptic density-95, discs large, zona occludens 1 (PDZ) domains and we show here that via its PDZ domains, X11α interacts with a novel transcription factor, fibrinogen silencer binding protein (FSBP). Moreover, we demonstrate that an X11α/FSBP complex signals to the nucleus to repress glycogen synthase kinase-3β (GSK3β) promoter activity. GSK3β is a favoured candidate kinase for phosphorylating tau in Alzheimer's disease. Our findings reveal a new function for X11α that may impact on Alzheimer's disease pathogenesis.

Keywords

Alzheimer's disease; Tau; GSK3β; X11α; FSBP; Mint1; FE65

Introduction

Alzheimer's disease is characterised by two pathologies, amyloid plaques and neurofibrillary tangles. Amyloid plaques contain deposits of Aβ peptide which is derived by proteolytic cleavage from APP; neurofibrillary tangles comprise a hyperphosphorylated form of the microtubule-associated protein tau [1].

X11α (also known as munc-18-interacting protein-1; mint1) is a member of a family of adaptor proteins that also includes X11β and X11γ [2]. X11α binds to APP via a centrally located PTB domain and its overexpression inhibits Aβ production [3-6]. In addition to its PTB domain, X11α also contains a number of other protein-protein interaction domains including two C-terminal PDZ domains through which it interacts with a number of other ligands [7-9]. Here,

Correspondence to: Kwok-Fai Lau, Tel. 852 26921106. Fax 852 26037732. kflau@cuhk.edu.hk. or Chris Miller, Tel. 44(0)2078480393. Fax 44(0)2077080017. chris.miller@kcl.ac.uk.

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we demonstrate that via its PDZ domains, X11α also binds to the transcription factor, FSBP. Furthermore, we show that an X11α-FSBP complex acts to repress GSK3β gene promoter activity. GSK3β is a favoured candidate kinase for phosphorylating tau in Alzheimer's disease [10]. Thus, our results provide a new function for X11α and one that might impact on Alzheimer's disease pathogenesis.

Methods

Plasmids

Plasmids for X11α and FE65 were as described [7,8,11]. Human FSBP cDNA was generated by PCR from a brain cDNA library and cloned into pCMV-Tag2 (Stratagene) for FLAG-tagged expression in cells. X11α PDZ1 and PDZ2 domain "wrecking" mutants were generated by mutating the key ILGV (residues 666-669 in PDZ1) and QLGF (residues 778-781 in PDZ2) to alanine. A glutathione *S*-transferase (GST)-FSBP fusion protein (GST-FSBP) was created by subcloning FSBP into pGEX-5X-1 (GE Healthcare). Plasmids for quantifying APP intracellular domain (AICD)-dependent transcription and GSK3β promoter activities were as described [11,12]. Plasmids for X11α and FSBP fused to GAL4 DNA binding domain (GAL4DNAbd-X11α and GAL4DNAbd-FSBP) were created by subcloning of the X11α and FSBP cDNAs into pM GAL4 fusion/expression vector. Transfection efficiency control vector plasmid pRL-TK was from Promega.

Yeast two-hybrid screen

The yeast two-hybrid library screen was performed as described [8].

Antibodies

A rabbit polyclonal FSBP antibody was generated by immunization with GST-FSBP. X11α was detected using a rabbit polyclonal antibody [7] or via the myc tag with antibody 9B11 (Cell Signalling Technology). Presenilin was detected using PS1Ab [7]; anti-c-Jun rabbit polyclonal antibody was from Santa Cruz; anti-α-tubulin (DM1A) and anti-FLAG (M2) antibodies were from Sigma.

Cell transfection and analyses

CHO and COS7 cells, and primary rat cortical neurons were cultured, transfected and analysed by SDS PAGE and immunoblotting, and by immunostaining as described previously [11,13, 14]. Likewise, cytosolic, membrane and nuclear fractions, and GST fusion pull-down and immunoprecipitation assays were as described [7,11].

Luciferase reporter assays

Luciferase reporter assays for measuring GAL4- and GSK3β promoter-dependent transcription in transfected CHO cells were as described [11]. Transfection efficiencies were determined by co-transfection of pRL-TK plasmid which expresses *Renilla* luciferase. Statistical analyses involved one-way ANOVA with LSD post hoc test.

Northern analyses

Northern analyses were performed on a human multiple tissue Northern blot (Clontech).

Results

To identify proteins that interact with X11α, we screened a human brain yeast two-hybrid library with the X11α PDZ domains as "bait"; PDZ domains are known mediators of protein-protein interactions [8]. We isolated a cDNA that encoded the C-terminal 189 amino acids of

the uncharacterised transcription factor FSBP; FSBP has been described as down-regulating transcription of the γ-chain of human fibrinogen gene (Genbank AF007866). Northern blots showed that FSBP was encoded by a major mRNA species of approximately 7 kb that was expressed in multiple tissues including brain; a less abundant 9.5 kb species was also detected (Fig. 1A).

To confirm that X11α and FSBP interact and to determine which X11α PDZ domain mediates the interaction, we used GST fusion proteins containing both X11α PDZ domains (X11α-PDZ1&2), the N-terminal PDZ domain (X11α-PDZ1) and the C-terminal PDZ domain (X11α-PDZ2), and used these as "baits" in pull-down assays from FLAG-FSBP transfected CHO cells. X11α-PDZ1&2 but not X11α-PDZ1 or X11α-PDZ2 alone interacted with FSBP in these assays (Fig. 1B). We also created GST-X11α-PDZ1&2 domain "wrecking" mutants by altering key residues as described [9] and tested these for their ability to bind FLAG-FSBP. Mutants in which either PDZ1 (X11α-PDZ1*&2), PDZ2 (X11α-PDZ1&2*), or both PDZ1 and PDZ2 together (X11α-PDZ1*&2*) were tested and again, X11α-PDZ1&2 but not X11α-PDZ1*&2, X11α-PDZ1&2* nor X11α-PDZ1*&2* bound to FLAG-FSBP (Fig. 1C).

We also generated a GST-FSBP fusion protein and used this in pull-down assays from X11α transfected CHO cells. GST-FSBP but not GST alone bound X11α in these assays (Fig. 1D). Finally, as FSBP is a nuclear protein (see below; Fig. 2) we tested whether X11α binds to FSBP in immunoprecipitation assays from the nuclear fraction of X11α/FLAG-FSBP co-transfected cells and cortical neurons. FLAG-FSBP was immunoprecipitated from FLAG-FSBP + X11α or X11α-only transfected CHO cells by the M2 anti-FLAG antibody and the lysates probed with an anti-X11α antibody for the presence of X11α. This revealed that X11α was present specifically in the FLAG-FSBP + X11α lysates (Fig. 1E). Similarly, endogenous FSBP and X11α were co-immunoprecipitated from nuclear lysate of cortical neurons. (Fig. 1F). Thus X11α binds FSBP in a variety of biochemical assays and this interaction requires both X11α PDZ domains.

We next examined the subcellular location of X11α and FSBP in transfected COS-7 cells. We found that FSBP was present within the nucleus (Fig. 2A) which is consistent its GenBank description as a gene silencer binding protein. X11α labelling was most intense in perinuclear regions consistent with previous reports that demonstrated it to be associated with Golgi and ER [15]. However, we observed a weaker but nevertheless consistent signal for X11α in nuclei (Fig. 2A). To confirm the presence of X11α in nuclei by another method, we prepared cytoplasmic, membrane and nuclear fractions from COS-7 cells transfected with X11α, FSBP or X11α + FSBP and analysed expression of each protein by immunoblotting. FSBP was present exclusively in nuclei but while the majority of X11α localised to the cytoplasmic/membrane fractions, a proportion (approximately 5%) was also present in nuclei (Fig. 2B). Co-transfection of FSBP with X11α did not noticeably alter the relative proportions of X11α in each compartment (Fig. 2B).

In a similar fashion, we examined the distribution of endogenous X11α and FSBP in cultured cortical neurons by preparing cytoplasmic/membrane and nuclear fractions and probing for each protein on immunobots. Again, FSBP was present only in the nuclear fraction but X11α localised to both cytoplasmic/membrane and nuclear factions (Fig. 2C).

The C-terminal intracellular domain of APP (AICD) has been shown to translocate to the nucleus with another PTB bearing adaptor protein FE65 and this AICD/FE65 complex has been proposed to regulate gene transcription [16]. We therefore enquired whether an AICD/X11α/FSBP complex might regulate transcription in a similar fashion. To do so, we monitored the effects of X11α and/or FSBP on AICD dependent transcription in transfected CHO cells. This involved use of a previously described assay in which a GAL4 DNA-binding domain-

AICD fusion protein drives expression of a GAL4UAS luciferase reporter [12,16]. Transfection of FE65 which is known to stimulate transcription in this assay was used as a positive control [12,16]. The presence of FE65 but not X11α stimulated transcription of the reporter as previously described [16] but neither FSBP nor X11α + FSBP stimulated transcription (Fig. 3A).

In variations of the above assays, we also prepared GAL4 DNA-binding domain-X11α and -FSBP fusions and tested whether these were capable of activating transcription of the GAL4UAS reporter either alone, or with co-transfected X11α or FSBP. However, we detected no stimulation of transcription of the GAL4UAS reporter in any assay (Fig. 3B). Thus, unlike AICD and FE65, X11α and FSBP appear not to stimulate transcriptional activity in GAL4 model systems.

One target for AICD dependent transcription is the GSK3β gene [11,17]; GSK3β is a favoured candidate kinase for phosphorylating tau in Alzheimer's disease [10]. Since X11α binds to AICD and since we found it in the nucleus in association with FSBP, we therefore enquired whether X11α and/or FSBP might influence transcriptional activity of the GSK3β gene. To do so we used a previously described GSK3β promoter-luciferase reporter (GSK3β promoter-Luc) [11]. Remarkably, we found that X11α and X11α + FSBP significantly inhibited GSK3β promoter activity by approximately 30% and 50% respectively; FSBP alone had no effect on transcription (Fig. 3C). Since FSBP but not X11α is expressed in non-transfected CHO cells [7], these results are consistent with the notion that the effect of X11α in X11α-only transfected cells is due to the presence of endogenous FSBP, and that the lack of effect of FSBP in FSBP-only transfected cells is due to the complete absence of X11α.

AICD has been shown to stimulate GSK3β promoter activity [11,17] and so we considered whether the repression effect of X11α and FSBP was via some influence on APP signalling. We tested the effect on GSK3β promoter activity of a mutant of X11α (involving a phenylalanine^{608} to valine substitution in the PTB domain) that cannot bind APP [18]. This mutant behaved identically to wild-type X11α (Fig. 3C). In contrast, mutation of PDZ1 and PDZ2 to abolish binding to FSBP (see Fig. 1C), completely eliminated the effect of X11α/FSBP on GSK3β promoter activity (Fig. 3C). Thus, X11α and FSBP repress GSK3β promoter activity and this inhibition requires an interaction between the two proteins but is not dependent upon binding of APP to the X11α PTB domain.

Discussion

Here, we show that via its two PDZ domains, X11α binds to the transcription factor FSBP and that FSBP and a proportion of X11α are present within nuclei. Nuclear FSBP is in agreement with its description as a repressor protein (Genbank AF007866) and others have also shown the presence of X11 proteins in nuclei [19]. Additional data suggests a role for X11α in nuclear signaling since it binds to CASK via N-terminal sequences [15] and CASK can function as a transcriptional co-activator of T-element containing genes with Tbr-1 [20]. Thus, our findings that FSBP and a proportion of X11α are present within nuclei are consistent with other studies.

APP and Fe65 have been shown to signal to the nucleus to stimulate expression of responsive genes and X11α has an inhibitory role on APP/FE65 signalling [21]. We show here that an X11α/FSBP complex is unable to stimulate transcription of an AICD dependent reporter and that neither X11α or FSBP are transcriptionally competent in GAL4 dependent systems. Rather, we found that X11α and FSBP together inhibited transcription of the GSK3β promoter. There is evidence that the GSK3β gene is one target for APP/FE65 signalling [11,17]. However, our results indicate that repression of the GSK3β promoter is distinct from this APP/FE65 signalling. Thus, while mutation of the X11α PDZ domains to eliminate binding to FSBP

abolished X11α/FSBP repression, mutation of the X11α PTB domain to eliminate binding to APP had no effect on GSK3β promoter activity. Likewise, JIP1/APP transcriptional regulation is known to be distinct from that of FE65/APP [22]. Thus, there appears to be several different pathways by which APP and/or its associated proteins can regulate gene activity.

Binding of X11α to APP inhibits Aβ production and as such, it has been suggested that X11α may be protective against Alzheimer's amyloid pathology [3-6]. Since GSK3β is implicated in tau hyperphosphorylation [10], our finding that an X11α/FSBP complex inhibits GSK3β promoter activity suggest that it might also impact on Alzheimer's disease tau pathology.

Conclusion

We show that a proportion of X11α is present in the nucleus where it binds to the repressor protein FSBP and that an X11α/FSBP complex represses transcription of the GSK3β promoter. Our results reveal a novel role for X11α in nuclear signalling.

Acknowledgments

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References

1. Kurz A, Perneczky R. Neurobiology of cognitive disorders. Curr Opin Psychiatry 2009;22:546–551. [PubMed: 19644374]
2. Miller CC, McLoughlin DM, Lau KF, Tennant ME, Rogelj B. The X11 proteins, Abeta production and Alzheimer's disease. Trends Neurosci 2006;29:280–285. [PubMed: 16545469]
3. Lee JH, Lau KF, Perkinton MS, Standen CL, Shemilt SJ, Mercken L, et al. The neuronal adaptor protein X11alpha reduces Abeta levels in the brains of Alzheimer's APPswe Tg2576 transgenic mice. J Biol Chem 2003;278:47025–47029. [PubMed: 12970358]
4. Sastre M, Turner RS, Levy E. X11 interaction with beta-amyloid precursor protein modulates its cellular stabilization and reduces amyloid beta-protein secretion. J Biol Chem 1998;273:22351–22357. [PubMed: 9712855]
5. Borg JP, Yang Y, De Taddeo-Borg M, Margolis B, Turner RS. The X11alpha protein slows cellular amyloid precursor protein processing and reduces Abeta40 and Abeta42 secretion. J Biol Chem 1998;273:14761–14766. [PubMed: 9614075]
6. Mitchell JC, Perkinton MS, Yates DM, Lau KF, Rogelj B, Miller CC, et al. Expression of the neuronal adaptor protein X11alpha protects against memory dysfunction in a transgenic mouse model of Alzheimer's disease. J Alzheimers Dis. 2010 in press.
7. Lau KF, McLoughlin DM, Standen C, Miller CC. X11alpha and X11beta interact with presenilin-1 via their PDZ domains. Mol Cell Neurosci 2000;16:557–565. [PubMed: 11083918]
8. McLoughlin DM, Standen CL, Lau KF, Ackerley S, Bartnikas TP, Gitlin JD, et al. The neuronal adaptor protein X11alpha interacts with the copper chaperone for SOD1 and regulates SOD1 activity. J Biol Chem 2001;276:9303–9307. [PubMed: 11115513]
9. Setou M, Nakagawa T, Seog DH, Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor- containing vesicle transport. Science 2000;288:1796–1802. [PubMed: 10846156]
10. Lovestone S, Reynolds CH, Latimer D, Davis DR, Anderton BH, Gallo JM, et al. Alzheimer's disease-like phosphorylation of the microtubule-associated protein tau by glycogen synthase kinase-3 in transfected mammalian cells. Curr Biol 1994;4:1077–1086. [PubMed: 7704571]

11. Lau KF, Chan WM, Perkinton MS, Tudor EL, Chang RC, Chan HY, et al. Dexras1 Interacts with FE65 to Regulate FE65-Amyloid Precursor Protein-dependent Transcription. J Biol Chem 2008;283:34728–34737. [PubMed: 18922798]
12. Perkinton MS, Standen CL, Lau KF, Kesavapany S, Byers HL, Ward M, et al. The c-Abl tyrosine kinase phosphorylates the Fe65 adaptor protein to stimulate Fe65/amyloid precursor protein nuclear signaling. J Biol Chem 2004;279:22084–22091. [PubMed: 15031292]
13. Brownlees J, Yates A, Bajaj NP, Davis D, Anderton BH, Leigh PN, et al. Phosphorylation of neurofilament heavy chain side-arms by stress activated protein kinase-1b/Jun N-terminal kinase-3. J Cell Sci 2000;113:401–407. [PubMed: 10639328]
14. Kesavapany S, Lau KF, Ackerley S, Banner SJ, Shemilt SJ, Cooper JD, et al. Identification of a novel, membrane-associated neuronal kinase, cyclin-dependent kinase 5/p35-regulated kinase. J Neurosci 2003;23:4975–4983. [PubMed: 12832520]
15. Borg JP, Lopez-Figueroa MO, de Taddeo-Borg M, Kroon DE, Turner RS, Watson SJ, et al. Molecular analysis of the X11-mLin-2/CASK complex in brain. J Neurosci 1999;19:1307–1316. [PubMed: 9952408]
16. Cao X, Sudhof TC. A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. Science 2001;293:115–120. [PubMed: 11441186]
17. Kim HS, Kim EM, Lee JP, Park CH, Kim S, Seo JH, et al. C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3beta expression. Faseb J 2003;17:1951–1963. [PubMed: 12923068]
18. Borg JP, Ooi J, Levy E, Margolis B. The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. Mol Cell Biol 1996;16:6229–6241. [PubMed: 8887653]
19. Sumioka A, Saito Y, Sakuma M, Araki Y, Yamamoto T, Suzuki T. The X11L/X11beta/MINT2 and X11L2/X11gamma/MINT3 scaffold proteins shuttle between the nucleus and cytoplasm. Exp Cell Res 2008;314:1155–1162. [PubMed: 18201694]
20. Hsueh YP, Wang TF, Yang FC, Sheng M. Nuclear translocation and transcription regulation by the membrane- associated guanylate kinase CASK/LIN-2. Nature 2000;404:298–302. [PubMed: 10749215]
21. Biederer T, Cao X, Sudhof TC, Liu X. Regulation of APP-dependent transcription complexes by Mint/X11s: differential functions of Mint isoforms. J Neurosci 2002;22:7340–7351. [PubMed: 12196555]
22. Scheinfeld MH, Matsuda S, D'Adamio L. JNK-interacting protein-1 promotes transcription of Abeta protein precursor but not beta precursor-like proteins, mechanistically different than Fe65. Proc Natl Acad Sci USA 2003;100:1729–1734. [PubMed: 12563035]

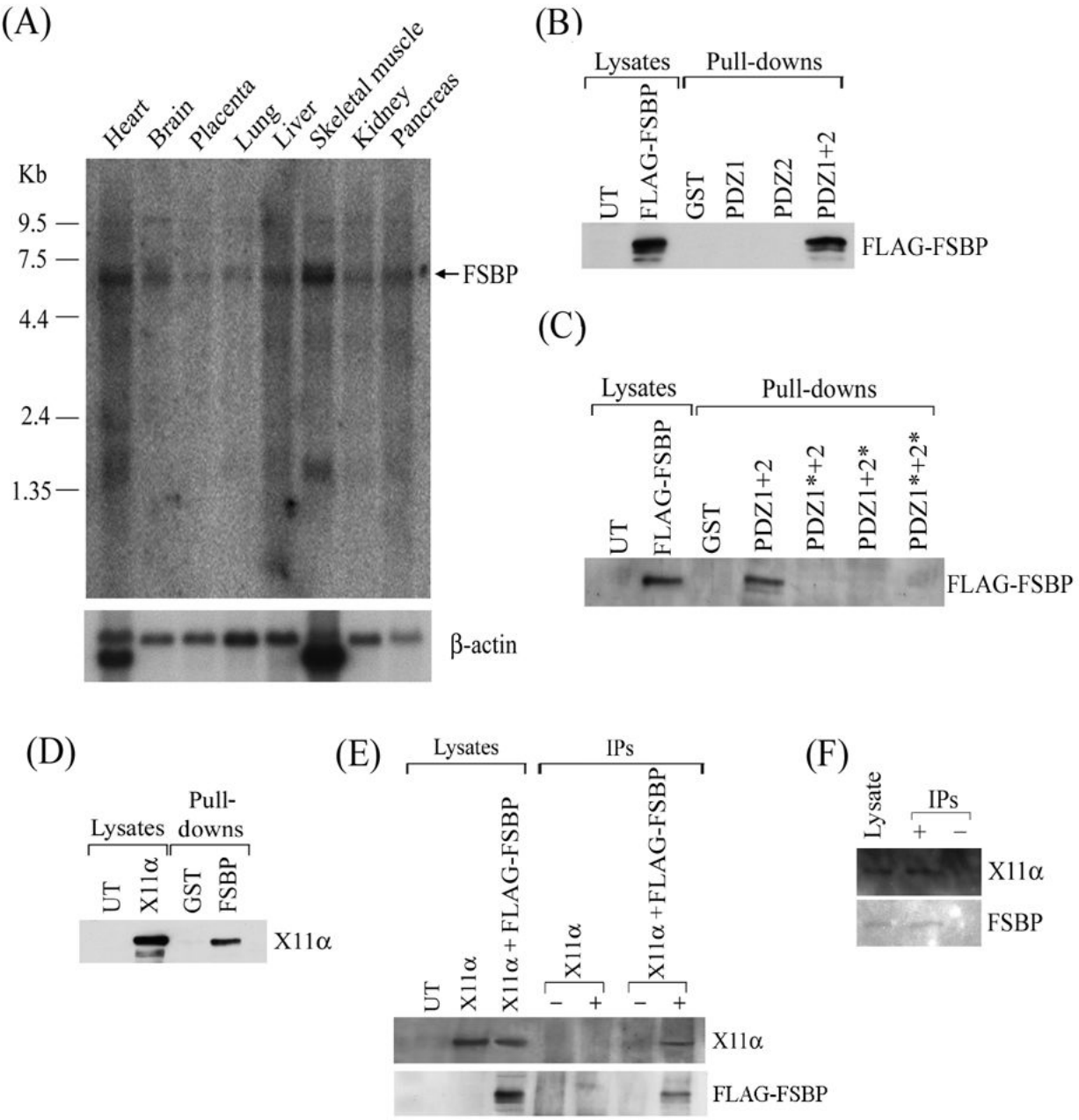

Figure 1.

X11α interacts with FSBP. (A) shows northern blots to demonstrate expression of FSBP. (B) GST pull-downs from FLAG-FSBP transfected CHO cells using GST, GST-X11αPDZ1, GST-X11αPDZ2 or GST-X11αPDZ1&2 "baits" as indicated. Only the X11αPDZ1&2 bait binds to FSBP. (C) shows GST pull-downs from FSBP transfected CHO cells using GST, GST-X11α PDZ1&2 or GST-X11α PDZ1&2 mutants in which the individual PDZ domains (PDZ1*&2, PDZ1&2*, PDZ1*&2*) were rendered non-functional by mutation (* indicates mutation of PDZ1 or PDZ2). Mutation of either or both PDZ domains abolished binding to FLAG-FSBP. (D) shows GST pull-downs from X11α transfected cells using GST or GST-FSBP as baits. (E) shows co-immunoprecipitation of FSBP with X11α. FLAG-FSBP was immunoprecipitated from X11α or X11α + FLAG-FSBP transfected CHO cells with anti-FLAG. The samples

probed for X11α and FLAG-FSBP with the anti-X11α antibody and the M2 anti-FLAG antibody, respectively. (−) and (+) refer to absence or presence of the FLAG antibody in the immunoprecipitations. (F) shows endogenous co-immunoprecipitation of FSBP with X11α from the nuclear lysate of rat cortical neurons. FSBP was immunoprecipitated by the affinity purified anti-FSBP antibody. The samples were probed for X11α and FSBP by the anti-X11α antibody and anti-FSBP antibody, respectively. (−) and (+) refer to absence or presence of the FSBP antibody in the immunoprecipitations. In (B-F), samples of the lysate inputs are shown; also shown is a sample of untransfected cell lysates (UT) for comparison.

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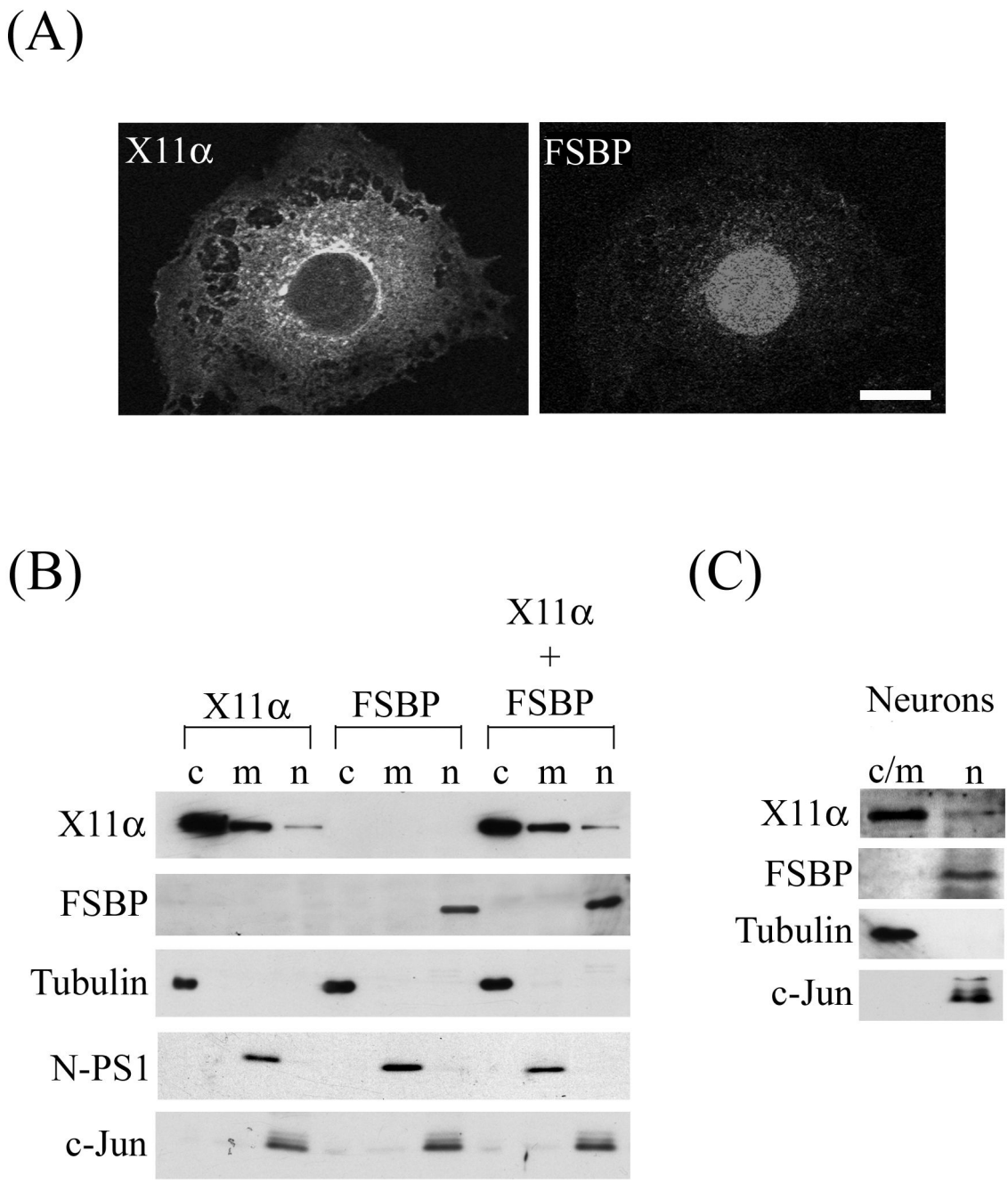

Figure 2.

FSBP is a nuclear protein and a proportion of X11α also locates to the nucleus. (A) shows confocal immunofluorescence labelling of COS-7 cells co-transfected with X11α (detected with rabbit anti-X11α) and FSBP (detected with mouse anti-FLAG). FSBP is present within the nucleus and whilst the majority of X11α is cytoplasmic, a proportion is also seen in the nucleus (scale bar is 10μm). (B) shows immunoblots of soluble cytoplasmic (c) cytoplasmic membrane (m) and nuclear (n) fractions of X11α, FSBP and X11α + FSBP transfected COS7 cells as indicated. FSBP was detected using the FLAG tag; X11α using rabbit anti-X11α. The samples were also probed for tubulin, presenilin-1 (N-PS1) and c-Jun as fraction markers. (C) shows immunoblots of combined cytoplasmic/membrane (c/m) and nuclear (n) fractions from

7 day old rat cortical neurons probed for endogenous FSBP and X11α with rabbit anti-FSBP/anti-X11α antibodies as indicated. Markers for cytoplasm/membrane (c/m) and nuclei (n) were tubulin and c-Jun respectively.

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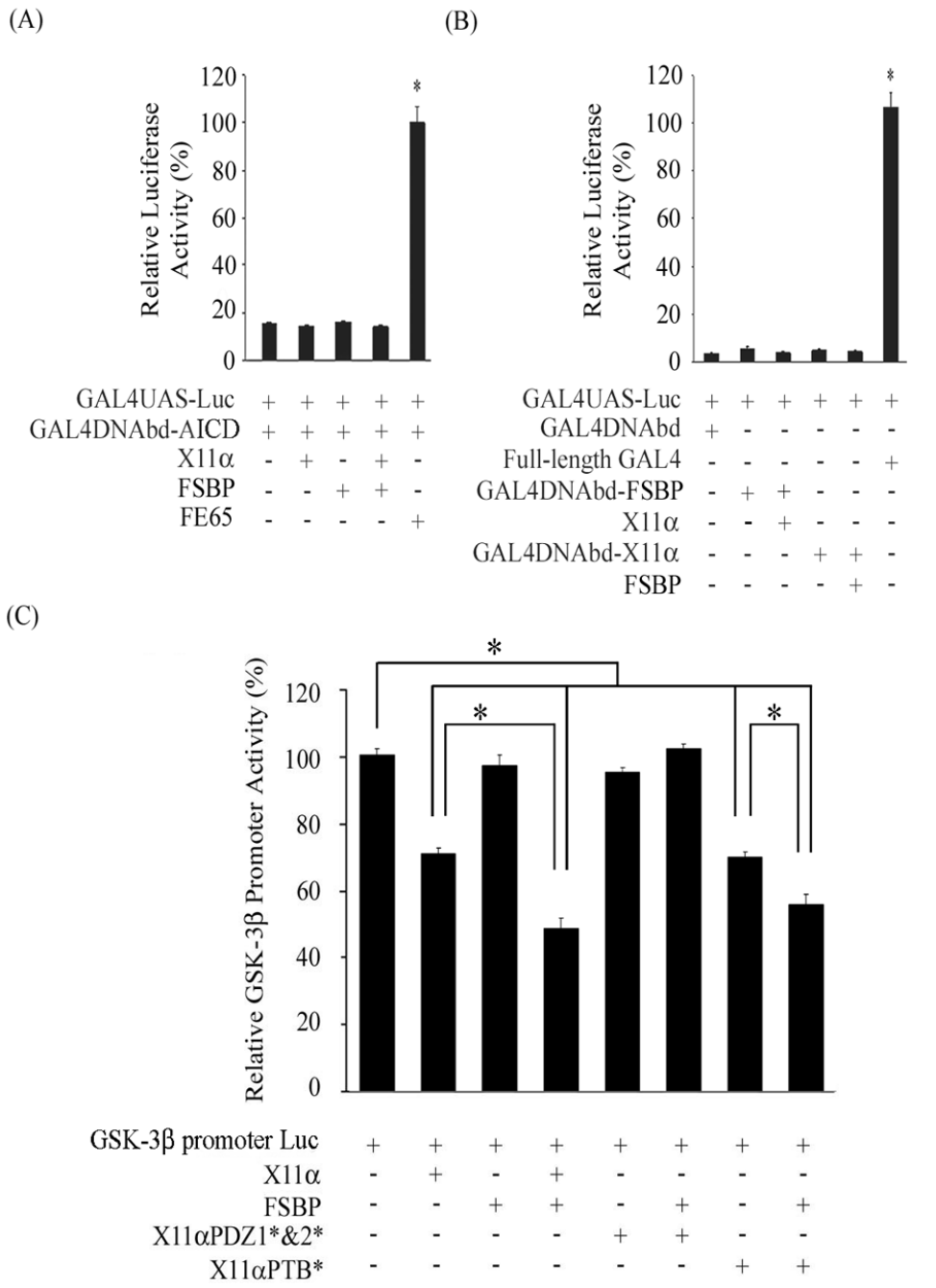

Figure 3.

X11α and FSBP do not influence GAL4-AICD dependent transcription and are not themselves transcriptionally competent when fused to the GAL4 DNA binding domain but instead together repress GSK3β promoter activity. (A) effects of X11α, FSBP and FE65 on transcription of a GAL4UAS dependent firefly luciferase reporter (GAL4UAS-Luc) together with a GAL4 DNA-binding domain-AICD fusion gene (GAL4DNAbd-AICD); only FE65 stimulates transcription. (B) inability of X11α or FSBP to activate GAL4-dependent transcription. Cells were transfected with GAL4UAS-Luc, and either GAL4 DNA-binding domain-FSBP or -X11α fusion genes (GAL4DNAbd-FSBP, GAL4DNAbd-X11α) alone or with additional FSBP/X11α. Full-length GAL4 and the GAL4DNAbd were used a positive and negative

controls. (C) repression of GSK3β promoter activity by X11α and FSBP. Cells were transfected with GSK3β promoter-firefly luciferase reporter (GSK3β promoter-Luc) and combinations of FSBP and X11α. In addition, the effects of mutating the X11α PDZ domains to abolish binding of FSBP (X11αPDZ1*&2*) or its PTB domain to abolish binding of APP (X11αPTB*) were also examined. X11α and X11α + FSBP significantly inhibit GSK3β promoter activity and the effect of X11α + FSBP was more potent than X11α. Mutation of the X11α PDZ but not PTB domains abolishes the effects of X11α/FSBP. * indicates significance $p<0.001$; n=12; error bars, SEM.