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18

Molecular Mechanism of the Involvement of the Susceptibility Genes, *DISC1, PACAP, TRAP1* and *Dysbindin* in Major Psychiatric Disorders Such as Schizophrenia, Depression and Bipolar Disease

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1. Introduction

No effective drugs are currently available for the treatment of mental diseases, primarily because the underlying mechanism of mental diseases have not been adequately explored at the molecular level. However, recent studies have examined several molecular cascades whose disturbances are associated with mental diseases such as schizophrenia, bipolar disease and major depression. The most common characteristics of these cascades us that they are all associated with neural circuit formation, suggesting that neurodevelopmental factors play a key role in the pathogenesis of mental diseases. The present review summarizes the available information on these molecular cascades and their association with mental disease.

2. Molecular mechanism of PACAP-stathmin1 dependent psychiatric disorders (Yamada et al., 2010)

Pituitary adenylate cyclase polypeptide (PACAP) is involved in multiple brain function such as neurotransmission and neural plasticity (Hashimoto et al., 2001; Vaudry et al., 2000). It also has a neurotrophic effect via three heptahekical G protein coupled receptors, one of which is specific for PACAP (PAC_1 receptor) and two others that are shared with vasoactive intestinal polypeptide ($VPAC_1$ and $VPAC_2$) (Hashimoto et al., 1993). Recently, mice that lack *Adcyap 1*, the gene encoding PACAP, (*Adcyap* $1^{-/-}$ mice) were developed (Hashimoto et al., 2001, Shintani et al., 2002). *Adcyap* $1^{-/-}$ mice display remarkable behavioral abnormalities providing evidence that PACAP plays a previously uncharacterized role in the regulation of

psychomotor behavior. In addition, previous association study reported that several single nucleotide polymorphism (SNPs) in the vicinity of the PACAP gene locus were associated with schizophrenia (Hashimoto, R. et al., 2007). However, although nothing was known about the mechanism of PACAP deficiency-induced psychiatric illness, we have clarified these mechanisms.

2.1 Down-regulation of PACAP expression induces up-regulation of stathmin1 expression in the dentate gyrus both *in vivo* and *in vitro*

Real-time PCR showed that stathmin1 mRNA was markedly increased in the dentate gyrus of *Adcyap 1*$^{-/-}$ mice. An increased in stathmin1 protein levels in the dentate gyrus of *Adcyap 1*$^{-/-}$ mice was confirmed by western blot analysis. These findings were confirmed also i*n vitro* using PC12 cells. PACAP stimulation of PC12 cells caused a decrease in stathmin1 mRNA levels after 3 h, and expression continued to decrease over the next 24 h (Fig. 1A). Stathmin1 protein levels also decreased in response to PACAP, which caused a dose-dependent decrease of stathmin1 mRNA levels. The decrease of stathmin1 expression caused by PACAP stimulation was slightly, but statistically significantly, inhibited by pretreatment with a PAC_1/$VPAC_2$ receptor antagonist (PACAP6-38). In addition, pretreatment with a p38 antagonist (SB202190) or an ERK antagonist (PD98059) also inhibited the PACAP-induced decrease of stathmin1 expression. Co-administration of SB202190 and PD98059 strongly inhibited the effect of PACAP, reflecting the key roles played by p38 and ERK in the PACAP signaling pathway. On the other hand, VIP did not decrease stathmin1 expression. These results indicate that PACAP regulates stathmin1 expression via the PAC_1 receptor in neurons of the dentate gyrus.

2.2 Up-regulation of stathmin1 induces abnormal axonal arborization in neurons of the dentate gyrus subgranular zone

In wild-type mice, cells expressing stathmin1 were preferentially located in the innermost part of the granular cell layer, the so-called subgranular zone (SGZ), where neurogenesis of granular cells occurs in adults. Two types of stathmin1 containing processes were found, namely; dendrites and axons. Immunoreactivity for stathmin1 was significantly increased in the SGZ neurons of *Adcyap 1*$^{-/-}$ mice, although the actual number of immunoreactive cells was similar in mutant and wild-type mice. . The number of dot-like immunoreactive fibers belonging to axons was significantly increased in the polymorphic layer of *Adcyap 1*$^{-/-}$ mice, compared with wild-type mice. In support of the *in vivo* datas, over-expression of stathmin1 in the hippocampal primary culture neurons caused dramatic changes of axon fibers. Arborization of axon fibers was markedly increased by stathmin1 over-expression compared with that in normal primary cultured neurons. The number of secondary neurites on axons was also increased following over-expression of stathmin1. These findings indicated that an increase in stathmin1 expression in SGZ neurons leads to abnormal axon arborization.

If PACAP directly regulates stathmin1 expression in vivo, SGZ neurons should express PAC_1. In fact, strong expression of PAC_1 mRNA was identified throughout the entire granular cell layer, including the SGZ. Furthermore, SGZ neurons expressed both stathmin1 protein and PAC_1 mRNA. These results show that PACAP inhibits stathmin1 expression via the PAC_1 receptor.

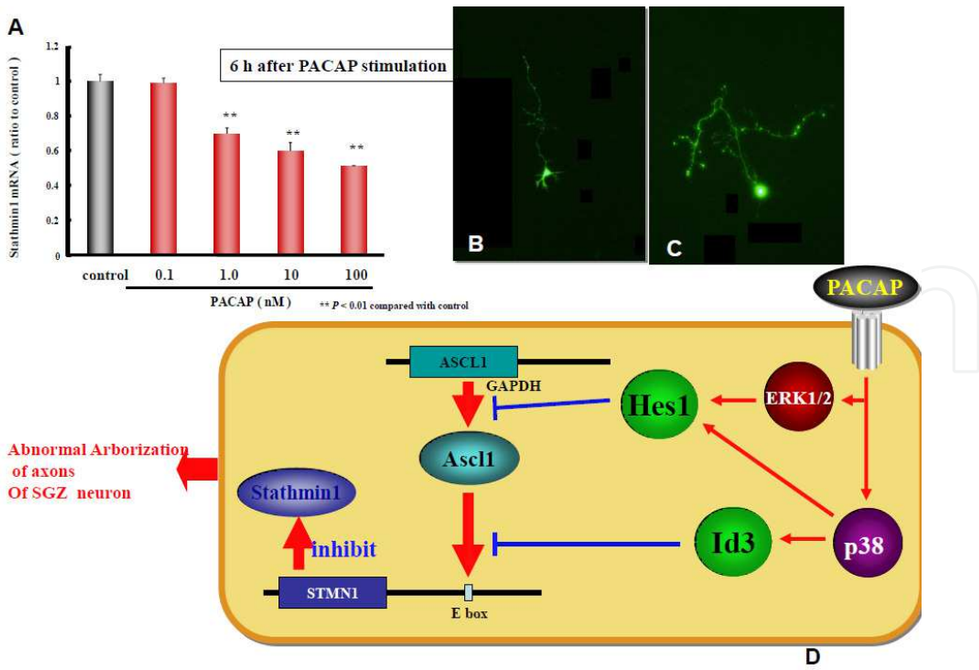


Fig. 1. PACAP-stathmin1 cascade
A: PACAP regulated stathmin1 expression via PAC1 in PC12 cells. Alteration of stathmin1 mRNA levels 24 h after PACAP treatment, at the indicated concentrations were quantified by real-time PRC. Data are expressed as mean ±SEM relative to control values (n=3, PACAP 1nM **P=0.0044, 10nM **P=0.0033, 100nM ***P=0.0003 compared with control. B,C; Stathmin1 over-expression in neurons caused abnormally pronounced arborization of axon fibers. Morphology of hippocampal primary neurons transfected with stathmin1. Over-expression of GFP (B) and GFP-stathmin1 (C). The neurons over-expressing stathmin1 have abnormally pronounced sprouting of axon fibers. D: Schematic drawing of the molecular pathway underlying PACAP regulation of stathmin1 expression. The schematic representation shows the pathway by which PACAP regulates the expression of stathmin1 by suppressing the function and expression of Ascl1 after increasing the expression of Hes1 and Id3 by activating ERK and p38.

2.2.1 Stathmin1 gene promoter activity is regulated by basic helix loop helix (bHLH) proteins via the E10 box

A BLAST search identified the genomic sequence of rat stathmin1 in a chromosome 5 contig. PCR amplification of a 1885bp genomic DNA fragment that consisted of 1561 nt upstream of the stathmin1 transcription start site (+1), exon1, and part of intron 1 (+325) was then performed. This fragment was sequenced and subcloned into a pGL3 luciferase reporter vector. This fragment was also analyzed for transcription factor-binding sites using the DNAsis program. The 1.8kbp rat stathmin1 5' genomic sequence contained 12 (E1-E12) putative E boxes (CANNTG), which are potential binding sites for bHLH proteins, including neuronal transcription activators. To investigate the promoter activity of stathmin1, we constructed several expression plasmids for the luciferase assay after transient transfection in PC12 cells. Constructs containing E10 (such as STMN1-1, STMN1-2 and STMN 1-3) showed a high level of luciferase activity compared with control cells, but

constructs containing a stathmin promoter lacking the E10 box (such as STMN1-4) did not show luciferase activity. Therefore, the E10 box was found to be a key motif that regulates stathmin1 expression through bHLH factors.

2.2.2 An activating bHLH protein, Ascl1, activates the stathmin promoter

Among activating bHLH proteins, Ascl1was found to activate the stathmin1 promoter. Co-transfection of PC12 cells with the stathmin1-promoter plasmid and the Ascl1 expression plasmid induced a dose-dependent increase in luciferase activity compared with cells transfected with the stathmin1 promoter plasmid alone. To examine whether endogenous Ascl1 protein could bind to the stathmin1 promoter sequence in PC12 cells, sheared chromatin was immunoprecipitated with an anti-Ascl1 antibody or with control IgG, followed by PCR amplification of the corresponding DNA regions using stathmin1 promoter specific primers. Analysis of amplified DNA showed that more sequences were amplified by primers flanking the E10 box than by primers flanking the E10-E11 boxes. In addition, co-localization of Ascl1 and stathmin1 in SGZ neurons was demonstrated by immunohistochemistry. These results established that endogenous Ascl1 protein binds to stathmin1 promoter and act as a major regulator of stathmin1 promoter activity.

2.2.3 The inhibitory bHLH proteins, Hes1 and Id3, showed increased expression after PACAP stimulation

As described above, PACAP inhibits stathmin1 expression. In addition, PACAP stimulation of PC12 cells caused an increase in the expression of the inhibitory HLH proteins, Hes1 and Id3 expression which belong to inhibitory HLH proteins. Inhibition of the PACAP signaling pathway, as through the inhibition of p38 and ERK, suppresses the effect of PACAP on stathmin1 expression. Moreover, the increase of Id3 mRNA levels in response to PACAP stimulation was inhibited by a p38 inhibitor (SB202190), but not by an ERK inhibitor (PD98059), while induction of Hes1 mRNA by PACAP stimulation was inhibited by both an ERK inhibitor and a p38 inhibitor. Co-administration of p38 and ERK inhibitors strongly inhibited thePACAP-induced induction of Hes1 mRNA. These findings showed that Hes1 expression is regulated by both the PACAP-ERK and PACAP-p38 pathways, whereas Id3 expression is mainly controlled by the PACAP-p38 pathway.

2.2.4 Hes1 and Id3 suppress stathmin1 promoter activity via Ascl1 inhibition

PC12 cells were co-transfected with a stathmin1 promoter plasmid and a Hes1 expression plasmid or an Id3 expression plasmid with or without an Ascl1 expression plasmid. Even without exogenous Ascl1 expression, a high level of luciferase activity was detected, owing to the action of the stathmin1 promoter. Expression of Hes1 or Id3 in these cells inhibited luciferase activity related to the stathmin1 promoter activity through endogenous Ascl1. In PC12 cells transfected with the stathmin1 promoter plasmid and the Ascl1 expression plasmid, luciferase activity was higher than that in PC12 cells without the Ascl1 expression plasmid. Id3 expression in these cells inhibited the up-regulation of stathmin1 promoter luciferase activity, while Hes1 expression failed to reduce the luciferase activity induced by exogenous Ascl1.These findings suggested that Id3 inhibits activation of the stathmin1 promoter by both exogenous and endogenous Ascl1, while Hes1 only blocked the effect of endogenous Ascl1. Thus, it is likely that Id3 regulates Ascl1 at the protein level, while Hes1 regulates Ascl1 transcription. If so, inhibition of Hes1 expression should increase the

transcription of Ascl1. In fact, the up-regulation of Hes1 in PC12 cells by PACAP stimulation led to inhibition of Ascl1 expression. In addition, a reduction of Hes1 expression also resulted in an elevation of Ascl1 expression to 1.2 fold the control level. These results indicate that Ascl1, which controls stathmin1 expression, was functionally regulated by Id3 and quantitatively regulated by Hes1, in response to PACAP signaling.

2.3 Role of the PACAP-stathmin1 cascade in psychiatric disorders

As described above, PACAP inhibits stathmin1 expression. In addition, over-expression of stathmin1 causes abnormal axonal arborization (Fig. 1B,C), indicating that stathmin1 regulates the maturation of neurons and neural circuit formation. Furtehrmore, *Adcyap 1*$^{-/-}$ mice are known to show behavioral abnormalities, some of which might have potential relevance to mental disorders such as schizophrenia (Hashimoto et al.,2001; Shintani et al., 2002), and several SNPs in the vicinity of the *PACAP* gene locus are associated with schizophrenia (Hashimoto et al., 2007). If so, stathmin1 expression should be altered in the brain of patients with schizophrenia. Our RT-PCR study showed that stathmin1 mRNA levels were significantly increased in schizophrenic patients compared with age-matched controls. In contrast, stathmin1 was not significantly increased in the brains of patients with bipolar disorder.

3. Mechanism of PACAP-DBZ/DISC1 dependent psychiatric disorders (Hattori et al.,2007; Katayama et al., 2009)

DBZ (DISC1-binding zinc finger protein) was found as a DISC1(disrupted-in schizophrenia 1)-interacting molecules by yeast-2-hybrid screening of a complementary DNA (cDNA) library. Subsequent co-immunoprecipitation studies and yeast-2 hybrid assays showed that amino acids 348-597 of DISC1 act as the DBZ binding region, which indicates that the regions of DISC1 near the translocation breakpoint (amino acid 598) participate in the interaction with DBZ. DISC1 and DBZ co-localize diffusely in the cytoplasm and centrosome, and are involved in neurite extension. PACAP regulates the association between DISC1 and DBZ (for details on DISC1, see the chapter 6).

3.1 DISC1-DBZ interaction inhibits the neurite outgrowth (Fig.2)

DBZ mRNA was expressed exclusively in the brain, but was not expressed in peripheral tissues. To examine the functional role of the DISC1-DBZ interaction, PC12 cells stably expressing DISC1-HA and mock-transfected cells were infected with Adv-DBZ-GFP or Adv-GFP for 24 h. Immunoprecipitation and western blot analysis confirmed the over-expression of DBZ-GFP and DISC1-HA as well as the association between these 2 molecules. Over-expression of both proteins in PC12 cells caused a significant reduction in the number of neurite bearing PC12 cells after PACAP stimulation. However, over-expression of either DBZ or DISC1 alone had no effect. The region of DBZ encompassing amino acids 152-301 interacts with DISC1. PC12 cells were transiently transfected with DBZ (152-301)-IRES-GFP or with GFP alone and treated with PACAP (100nM) for 48 h. The cells expressing DBZ (152-301) had a shorter neurite length than cells expressing GFP alone. Under these conditions, no significant change of apoptosis was detected and the number of transfected cells was similar. The effect of DBZ (152-301) on neurite growth was also confirmed in primary cultured hippocampal neurons.

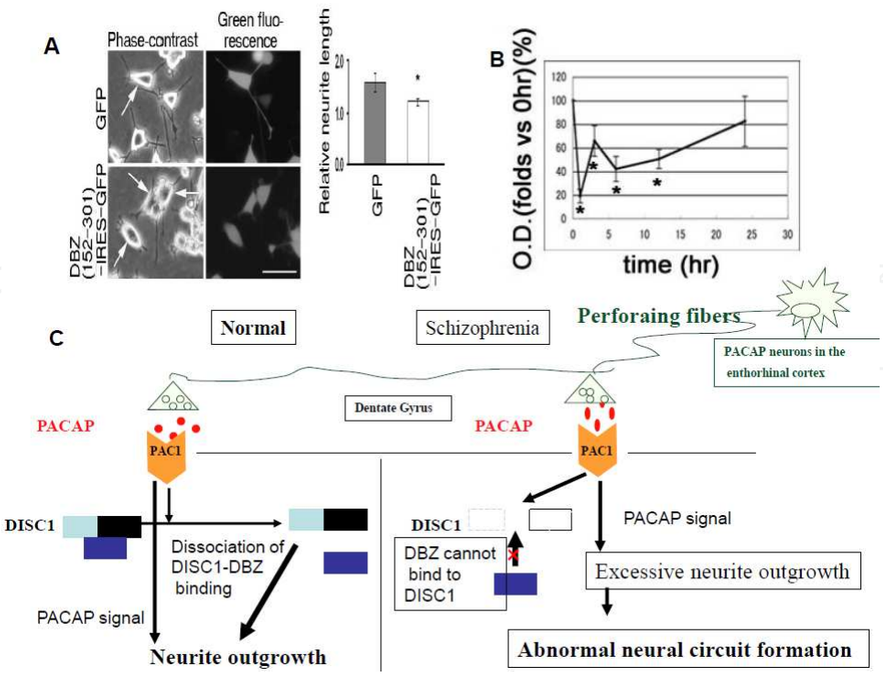


Fig. 2. A: Neurite outgrowth was inhibited by the DISC1-binding domain of DBZ [DBZ(152-301)-IRES-GFP]. PC12 cells were transfected with DBZ (152-301)-IRES-GFP or GFP alone at 2 days after plating. After 24 h , cells were starved of serum for 4h and treated with 100nM PACAP for 48h. Phase-contrast and fluorescence microscopy images are shown. Diagrams display neurite length relative to cell body diameter of transfected PC12 cells. B: PACAP-induced transient inhibition of the endogenous DISC1-DBZ interaction in PC12 cells. Immunoprecipitation and western blot analysis (with anti-DISC1 or anti-DBZ antibodies) of PC12 cells lysates collected at the indicated times after stimulation with 100nM PACAP. Immunoprecipitaes obtained with an anti-DBZ antibody incubated with 5% of each lysates (5% input), were subjected to western blot analysis with the same antibody. Quantitation of relative band densities for DISC1 co-immunoprecipitated with DBZ, as well as for total DISC1 or DBZ protein, was performed by scanning densitometry. Data were expressed as the mean ±SEM. of at least 3 independent experiments. *P<0.05 vs control (Student's t-test). C: A possible PACAP-DISC1/DBZ pathway . In the normal brain, PACAP leads to the temporal dissociation of the binding between DISC1 and DBZ , which in turn leads to normal neurite outgrowth. In the brain of schizophrenia patients, DBZ cannot bind to DISC1, and the PACAP pathway may proceed without inhibition, resulting in the formation of an abnormal neural circuit.

3.2 PACAP regulates DISC1-DBZ binding (Fig. 2)

Exposure to PACAP (100nM) increased the expression of endogenous DISC1 in PC12 cells by about 50% after 24 h, whereas it had no effect on DBZ expression. PACAP has a marked influence on the interaction between DBZ and DISC1. The co-immunoprecipitation of DISC1 and DBZ from PC12 cell lysates was reduced by approximately 80% 1h after treatment of cells with PACAP (100nM). However, this reduction was transient and there was a gradual return to control level by 24 h after treatment. Addition of an ERK inhibitor 24 h after PACAP treatment inhibited the rebinding of DBZ to DISC1, while an inhibitor of adenyl

cyclase failed to influence the DISC1-DBZ interaction, showing that PACAP regulates DISC1-DBZ binding through the ERK cascade but not through the cAMP cascade.

3.3 Role of the PACAP-DBZ cascade with regard to PACAP-DBZ/DISC1 dependent psychiatric disorders (Fig.2)

The findings described above show that the DISC1-DBZ interaction inhibits neurite out growth, and PACAP dissociates this interaction, resulting in neurite outgrowth. The involvement of the DISC1-DBZ interaction and PACAP in neurite outgrowth suggests that these molecules should be expressed in the early ontogenetical stages. In fact, a marked elevation of both DBZ and PAC_1 (PACAP receptor) expression was observed during the perinatal stage (Hattori et al. in preparation). DISC1 shows a high level of expression in the developing cortex and hippocampus (Honda et al.,2004). In addition, down-regulation of DBZ caused a delay in the migration of cortical neurons (Sato et al., personal communication) and disturbance of cilia formation (Kumamoto et al., in preparation). The DISC1-DBZ interaction which is regulated by PACAP has therefore been shown to be involved in neurite out growth and the migration of neurons. In the brain of patients with PACAP-dependent psychiatric disorders, dissociation of the binding between DISC1 and DBZ does not occur and neurite extension may be inhibited. On the other hand, in brains in which translocation of DISC1 occurs, DBZ is unable bind to DISC1 and the dissociation of DISC1 from DBZ may not be induced, which results in an immature neural circuit.

4. Molecular mechanism of Dysbindin-MARCKS cascade dependent psychiatric disorders (Okuda et al., 2010)

Studies of postmortem brain tissue showed decreased Dysbindin (dystrobrevin binding protein 1; DTNBP1) protein and mRNA levels in patients with schizophrenia compared with controls (Bray et al., 2005; Talbot et al., 2004; Weickert et al., 2004). Chronic treatment of mice with antipsychotics did not affect the levels of Dysbindin protein and mRNA expression in their brains (Chiba et al., 2006; Talbot et al., 2004), suggesting the lower levels of Dysbindin protein and mRNA found in the postmortem brains of schizophrenia patients is not likely to be a simple artifact of antemortem drug treatment. In addition, several studies suggested that diverse high-risk SNPs and haplotypes could influence Dysbindin mRNA expression (Bray et al., 2005; Talbot et al., 2004; Weickert et al., 2004). These data indicate that the *Dysbindin* gene may confer susceptibility to schizophrenia through reduced Dysbindin expression. However, the molecular mechanisms underlying the effect of decreased Dysbindin expression on vulnerability to schizophrenia remain unknown.

4.1 Dysbindin- myristoylated alanin-rich protein kinase C substrate (MARCKS) cascade (Okuda et al., 2010)

4.1.1 Dysbindin exists within the nucleus in addition to the cytoplasm

Cell fractionation experiments using Dysbindin-FLAG-overexpressing HEK293 cells showed that Dysbindin exists mainly in the cytosol while a small amount is present in the nucleus. Immunohistochemical analysis also revealed that Dysbindin is localized mainly in the cytoplasm with a perinuclear high density region. However, a faint immunoreaction was seen within the nucleus. Furthermore, pretreatment with leptomycine-B(LPB), which inhibits the export of proteins from the nucleus to the cytoplasm, caused a slight increase of

Dysbindin and its nuclear localization. These findings show that the Dysbindin protein is shuttled between the nucleus and the cytoplasm.

4.1.2 Dysbindin binds to the transcription factor NF-YB

Using yeast 2-hybrid screening, several transcriptional factors including nuclear transcription factor Y beta (NF-YB), were identified as candidates for interaction with Dysbindin. NF-YB belong to a family of CCAAT-binding transcription factors that are important for the basal transcription of a class of regulatory genes and are involved in cellular reactions. HEK293T cells which express NF-YB endogenously were transfected with expression vectors for Dysbindin-V5 and subjected to immunoprecipitation to confirm the Dysbindin-NF-YB interaction. In addition, the Dysbindin-NF-YB interaction was shown in lysates from SH-SY5Y cells, which express both Dysbindin and NF-YB endogenously, as well as adult mouse brain lysates.

4.1.3 Downregulation of Dysbindin causes up-regulation of the expression level of MARCKS

The interaction between Dysbindin and NF-YB suggests that Dysbindin may be functionally involved in the transcription of genes regulated by NF-YB. We therefore screened for genes displaying altered expression by means of a DNA chip, using RNA extracts from the Dysbindin or NF-YB knockdown human neural cell line, SH-SY5Y. Among them identified, we focused on MARCKS, because protein kinase C has been involved in psychiatric diseases and because the promoter region of the *MARCKS* gene has the CCAAT binding motif specific for NF-YB.

The effect of Dysbindin on MARCKS *in vitro* was confirmed *in vivo* by examining the expression of the MARCKS protein product in the hippocampus of Dysbindin knockout mice with advanced age and comparing the levels with those of wild-type mice. In wild-type mice, a peak in MARCKS protein expression in the hippocampus was detected on postnatal days 15 and 20, and with a marked decrease in expression levels over time. However, this decrease was not observed in Dysbindin knockout mice, suggesting that down-regulation of Dysbindin may enhance the transcription of the MARCKS protein.

Chromatin immunoprecipitation analysis using SH-SY5Y cells over-expressing Dysbindin-Flag, was performed to explore the possibility that the Dysbindin-NF-YB complex could affect the transcription of *MARCKS via* interaction with the promoter region of *MARCKS*. PCR products from the chromatin immunoprecipitates suggested that Dysbindin and NF-YB simultaneously interact with the promoter region of *MARCKS*. These results indicate that the Dysbindin-NF-YB complex interacts with the promoter region of the *MARCKS* gene resulting in inhibition of MARCKS transcription.

4.1.4 The transcriptional level of the *MARCKS* gene is regulated by Dysbindin via the NF-YB binding motif, CCAAT-2

The 5'-UTR region of the *MARCKS* gene has 2 kinds of CCAAT sequences, namely; one CCAAT motif located between residues -1152 and -700 (CCAAT-1) and one located between UTR -700 and -614 (CCAAT-2). Because NF-YB binds to the CCAAT motif to regulate the transcription of target genes，the role of the CCAAT motifs in the regulation of *MARCKS* transcription was examined by luciferase assay with 5 vectors containing shorter RNA probes (Fig. 3A); These vectors were UTR(1152)-Luc, UTR (953)-Luc, UTR (700)-Luc, UTR

(614)-Luc, and UTR (462)-Luc. The luciferase activity detected in SH-SY5Y cells expressing the UTR (1152)-Luc after retinoic acid stimulation was used as a baseline. In the cells transfected with UTR (953)-Luc containing both CCAAT sequence and UTR (700)-Luc containing CCAAT-1 sequence but lacking the CCAAT-2 sequence, luciferase activity remained at baseline level after stimulation with retinoic acid. However luciferase activity was markedly increased in cells expressing UTR (614)-Luc after retinoic acid stimulation. These results suggest that the CCAAT-2 motif plays an important role in the inhibition of M*ARCKS* transcription. Furthermore, SH-SY5Y cells transfected with UTR (462)-Luc lacking CCAAT-1, CCAAT-2 and SP1 region showed very low luciferase activity, indicating that the SP1 is indispensable for *MARCKS* transcription.

To confirm that the CCAAT-2 region is important for the regulation of MARCKS transcription, several probes were designed for the luciferase assay (Fig. 3Ｂ), namely; D1-UTR (1152)-Luc which lacks the CCAAT-2 motif and its downstream region including Sp1 from UTR (1152)-Luc, D2-UTR (1152)-Luc which lacks the SP1 region and the downstream sequence from UTR (1152)-Luc, D3-UTR (1152)-Luc which lacks only the sequence downstream of the SP1 region, D4-UTR (1152)-Luc which lacks only the CCAAT motif from UTR (1152)-Luc, and M-UTR(1152)-Luc, which has a point mutation in the CCAAT-2 motif. Luciferase activity was detected in SH-SY5Y cells transfected with each probe, using the activity in cells transfected with UTR (1152)-Luc as the baseline value. Cells transfected with M-UTR (1152)-Luc and those transfected with D4-UTR (1152)-Luc exhibited marked increases in luciferase activity, showing that the CCAAT-2 motif plays a key role in the inhibition of *MARCKS* transcription. Furthermore, cells expressing D1-UTR (1152)-Luc, D2-UTR (1152)-Luc or D3-UTR (1152)-Luc exhibited no luciferase activity. These findings suggest that the sequence downstream of the Sp1 region, and the Sp1 region itself, areindispensable for *MARCKS* transcription.

To confirm the involvement of Dysbindin in the altered MARCKS transcription levels via the CCAAT-2 motif, we compared the luciferase activity of UTR (1152)-Luc detected in Dysbindin knockdown cells with that of control cells. Knockdown of Dysbindin resulted in the up-regulation of luciferase activity in the UTR (1152)-Luc transfected cells. However, the effect of knockdown of Dysbindin knockdown on luciferase activity was not observed in D1-UTR (1152)-Luc transfected cells. These results suggest that Dysbindin regulates MARCKS transcription via the NF-YB binding motif CCAAT-2. On the other hand, the negligible levels of luciferase activity observed in cells transfected with probes lacking the sequence downstream of the Sp1 region, suggest that this this sequence is essential for *MARCKS* transcription. In fact, knockdown of Dysbindin caused the up-regulation of MARCKS expression (Fig. 3C).

4.2 Role of the Dysbindin-MARCKS cascade in psychiatric disorders (Fig. 3D)

SNPs in Dysbindin have been associated with intermediate cognitive phenotypes related to schizophrenia such as IQ and working and episodic memory, and a Dysbindin haplotype has been associated with higher educational attainment (Corvin et al., 2008; Donohoe et al., 2007). In addition, several papers show evidence of the involvement of Dysbindin in cognitive functions (Burdick et al., 2006; Zinkstok et al., 2007).

Furthermore, accumulating evidence suggests the involvement of Dysbindin in neurotransmission. At the cellular level, Dysbindin is located at both pre- and post-synaptic terminals., and is thought to be involved in postsynaptic density function and the

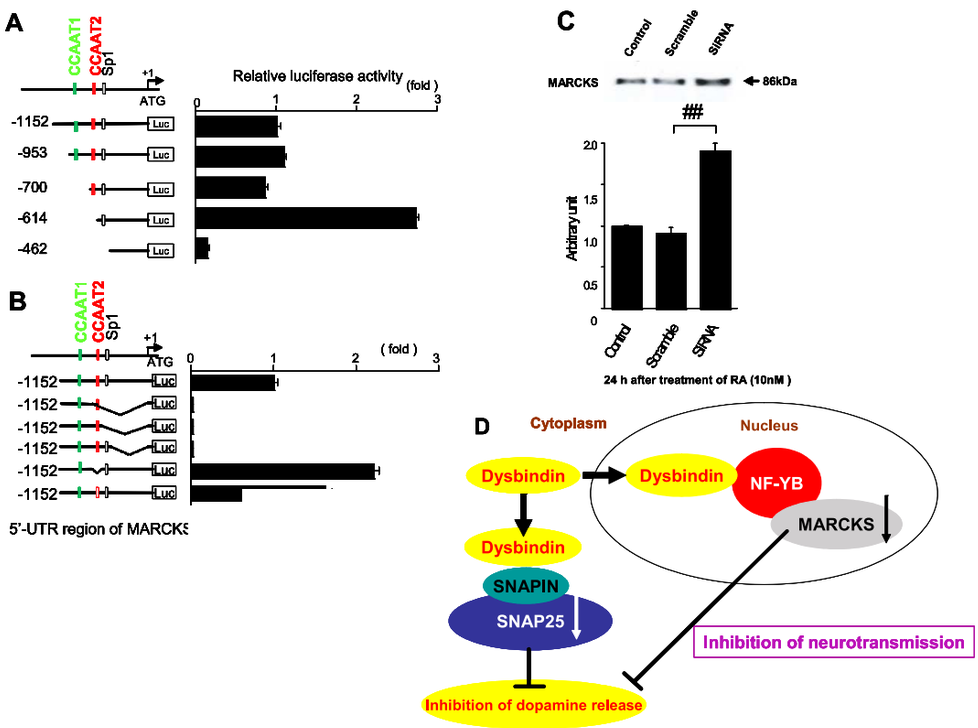


Fig. 3. A,B: Dysbindin regulates the transcription of MARCKSvia the CCAAT-2 sequence. A: The following 5 vectors contaiing shorter DNA probes were used for the luciferase assay; UTR(1152)-Luc, UTR (953)-Luc, UTR (700)-Luc, UTR (614)-Luc, and UTR (462)-Luc. Vectors were transfected into SH-SY5Y cells and luciferase activity was measured. The luciferase activity of UTR (1152) was used as the control. Columns and vertical bars denote the means ±SEM (triplicate independent experiments). B: The UTR (1152)-Luc vector and deletion or point mutations of the UTR (1152)-Luc vectors, [D10UTR(1152)-Luc], [D2-UTR (1152)-Luc], [D3-UTR (1152)-Luc], [D4-UTR(1152)-Luc] and [M-UTR (1152)-Luc], were transfected into SH-SY5Y cells and luciferase activity was measured. [D4-UTR(1152)-Luc], which lacks CCAAT-2, and [M-UTR (1152)-Luc], which has a point mutation in the CCAAT-2 sequence, showed increased luciferase activity. The luciferase activity of UTR (1152) was used as the control. Columns and vertical bars denote the means ±SEM(triplicate independent experiments). C: Dysbindin knockdown results in the up-regulation of MARCKS. SH-SY5Y cells were transfected with scrambled RNAi or siRNA for Dysbindin. Cell lysate of un-treated cells (cont.), scrambled RNAi-transfected cells (Scr.) and RNAi for Dysbindin-transfected cells (siRNA) were subjected to western blotting with an anti-MARCKS antibody. Columns and vertical bars denote the means ±SEM(triplicate independent experiments). Dysbindin knockdown cells exhibited significant up-regulation of MARCKS expression compared with control cells ($P<0.001$, Student's t-test). D: A possible Dysbindin pathway related to the regulation of dopamine release. In the cytoplasm of the nigrostriatal dopamine neurons, Dysbindin binds to Snapin. This binding inhibits the expression of SNAP25, which may suppress dopamine release. In the nuclei of these neurons, Dysbindin inhibits MARCKS expression via the binding to NF-YB. Reduction of MARCKS expression in dopaminergic neurons may causes the down-regulation of dopamine transport from the soma to the terminal. Thus Dysbindin inhibits dopamine release by 2 pathway.

trafficking of receptors (NMDA,GABAergic and nicotinic) (Sillitoe et al., 2003; Talbot et al., 2004). Over-expression of Dysbindin increases glutamate release from pyramidal neurons in cell culture, possibly because of its role in vesicular trafficking (Numakawa et al., 2004). Decreases in Dysbindin mRNA and protein levels have been reported in regions previously implicated in schizophrenia such as the prefrontal; cortex, midbrain and hippocampus (Talbot et al., 2004; Weickert et al., 2004). On the other hand, abnormal activation of nigrostriatal and mesolimbic dopaminergic systems is thought to be one of the most important etiologies for schizophrenia (Angrist & van Kammen, 1984; Creese et al., 1976; Lieberman et al., 1987; Seeman &Lee, 1975), suggesting a functional relationship between dopamine and Dysbindin. In support of this, midbrain dopamine neurons also contain Dysbindin (Kumamoto et al., 2006). Suppression of Dysbindin expression in PC12 cells resulted in an increase of the expression of SNAP25 which plays an important role in neurotransmitter release, and increased the release of dopamine. On the other hand, up-regulation of Dysbindin expression in PC12 cells showed a tendency to decrease the expression of SNAP25 and the release of dopamine. These findings show that Dysbindin inhibits dopamine release via down-regulation of SNAP25 expression (Kumamoto et al., 2006).

Thus, Dysbindin inhibits MARCKS expression and decreased expression of Dysbindin is characteristic of the schizophrenic brain. In addition, a decrease in Dysbindin levels up-regulates dopamine release. MARCKS influences neurotransmission *via* F-actin and vesicular transport *via* synaptic vesicles. The enhanced dopaminergic transmission produced by the lower expression level of Dysbindin may be partially attributed to activation of MARCKS. Thus, the impairment of neuronal transmission in the schizophrenic brain may be caused by alterations of MARCKS expression levels *via* changes in Dysbindin.

Sandy (*sdy*) mice that express no Dysbindin, showed behavioral abnormalities, which could be endophenotypes of schizophrenia (Feng et al., 2008). These mutant mice reportedly exhibit defective synaptic structure and function of CA_1 neurons (Chen et al., 2008), though the mechanism by which the loss of Dysbindin induces schizophrenia-like behaviors, remains unclear. Recently, we revealed that Dysbindin is involved in neural development through the regulation of the actin skeleton organization (Kubota et al., 2008). This study showed that knockdown of Dysbindin resulted in the aberrant organization of the actin cytoskeleton in SH-SY5Y cells. Furthermore, morphological abnormalities of the actin cytoskeleton were similarly observed in growth cones of cultured hippocampal neurons derived from sdy mice. In addition, a significant correlation was found between Dysbindin expression levels and the phosphorylation level of c-Jun N-terminal kinase (JNK), which is implicated in the regulation of cytoskeletal organization. These findings revealed that Dysbindin plays a key role in coordinating JNK signaling and actin cytoskeleton organization, which are required for neuronal development.

5. Mechanism of tumor necrosis factor receptor (TNFR) associated protein 1 (TRAP1)-N-cadherin alteration-induced psychiatric disorders

An increase in serum tumor necrosis factor-α (TNF-α) level is closely related to the pathogenesis of major depression (Irwin & Miller, 2007). The tumor necrosis factor receptor assoicated protein (TRAP1) was detected in whole brain lysates (Song et al., 1995). TRAP1 is a member of the heat shock protein 90 (HSP90) family and possesses ATPase activity, but lacks chaperone activity (Felts et al., 2000). However, the function and molecular mechanism

of the TNF-TRAP system remain unclear. In the following section, we will describe the evidence that TRAP1 regulates the expression of adhesion molecule (Kubota et al., 2009).

5.1 TRAP1 is widely expressed in neurons through the brain, including in regions known to be affected in patients with major depression

In situ hybridization histochemistry and immunocytochemistry revealed that TRAP1 mRNA and protein are broadly expressed in neurons throughout the gray matter of the brain and the spinal cord, including in regions known to be affected in patients with major depression patients, such as the medial prefrontal cortex, hippocampus and nuclei producing monoamine: the substantia nigra pars compacta, dorsal raphe nucleus and locus ceruleus (Nestler et al., 2002; Berton and Nestler, 2006). However, glial cells such as astrocytes and oligodendrocytes are devoid of TRAP1. In addition, punctate immunostaining of TRAP1 was detected in the cytoplasm, which is consistent with previously reported mitochondrial localization of TRAP1 in a cell culture (Felts et al., 2000).

5.2 TRAP1 regulates cell adhesion

A striking cell-scattering phenotype was observed in TRAP1 knockdown SH-SY5Y cells. Cells transfected with siTRAP1 were dispersed throughout the dish, compared to cells transfected with control siRNA, which grew in aggregates resembling untransfected SH-SY5Y cells (Fig. 4A,B). This phenomenon was detectable as early as 24 h after transfection and became more prominent by 72 h after transfection. Immunostaining of actin filaments in siRNA-treated cells showed no difference in cytoskeletal structure. Quantification of the percentage of cells with no inter-cellular contacts after staining for actin detected a 6.2-fold increase in cells transfected with siTRAP1(36%) compared to cells transfected with control siRNA (5.8%). The cell aggregation assay revealed that TRAP1 knockdown cells were characterized by decreased efficacy of cell-cell adhesion compared with control cells, suggesting an alteration in calcium-dependent cell adhesion is affected. These results strongly indicate that TRAP1 regulates downstream molecules crucial for cell adhesion.

5.3 N-cadherin is transcriptionally down-regulated in TRAP1 knockdown cells

Expression levels of cell adhesion molecules, including N-cadherin, are directly related to the cell-scattering phenomenon (Hayashida et al., 2006; Yasuda et al., 2007) and N-cadherin mediates calcium-dependent cell adhesion in neuronal cells (Takeuchi and Nakagawa, 2007) Our findings showed that N-cadherin levels were remarkably decreased throughout the cytoplasm of TRAP1 knockdown cells, including around the membrane where cell-adhesion takes place, compared to control cells (Fig. 4C,D). Immunoblotting experiments confirmed this finding, showing a significant decrease in N-cadherin expression in TRAP1 knockdown cells from as early as 24 h until at least 2 h after transfection. However, the expression level of β-catenin, which is involved in the regulation of cell-adhesion, was unaffected. These results suggest that the cell scattering phenotype detected in TRAP1 knockdown cell is at least partially mediated by a reduction of N-cadherin expression in those cells.

To exclude the possibility that cell viability or migration may contributes to the cell-scattering phenotype in TRAP1 knockdown cells, we examined cell viability by the 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and migration by the wound-healing assay. Although a slight decrease in cell viability was observed in TRAP1 knockdown cells compared to control cells 48 h after transfection or later, no changes were

detected 24 h after transfection, when the cell-scattering phenotype of TRAP1 was already observed. No significant changes in the rate of cell migration were observed.
To determine if down-regulation of N-cadherin induced by siTRAP1 occurs at the transcriptional level, N-cadherin mRNA levels were measured by real-time RT-PCR analysis 48 h after siRNA transfection, which showed that N-cadherin mRNA levels in TRAP1 knockdown cells were approximately 45% lower than in control cells. These results indicate that TRAP1 knockdown induces transcriptional down-regulation of N-cadherin.

5.4 E2F1, a putative transcription factor of N-cadherin, is down-regulated in TRAP1 knockdown cells

To determine the possible involvement of a transcription factor in down-regulation of N-cadherin in TRAP1 knockdown cells, a search DBTSS (Database of Transcriptional Start Sites) and TRANSFAC (The Transcription Factor Database) database was conducted. This search revealed a putative binding site for E2F1 in the promoter region of the N-cadherin gene. Immunoblot analysis and real-time PCR showed that E2F1 mRNA and protein levels were significantly decreased in TRAP1 knockdown cells (Fig. 4E), although the mRNA level of c-Myc, another representative transcription factor, was not affected, In addition, SH-SY5Y cells transfected with the N-cadherin-luciferase plasmid showed strong activity of the reporter, and this activity was suppressed in TRAP1 knockdown cells, mimicking the signaling cascade detected *in vitro*. Furthermore, exogenously transfected E2F1 showed a 7.5-fold induction of luciferase reporter activity relative to the control vector. These results indicate that E2F1 plays a regulatory role upstream of N-cadherin in TRAP1 knockdown cells.

5.5 Reduced phosphorylation of STAT causes down-regulation of E2F1 in TRAP1 knockdown cells

A recognition sequence for STAT3 is located 89bp upstream of the transcription initiation site of the *E2F1* gene. Upon activation, STAT3 proteins are tyrosine-phosphorylated, dimerize and translocate to the nucleus where the nuclear phospho-STAT binds to STAT recognition sites located in the promoter region of downstream genes to promote the transcription of those genes. In TRAP1 knockdown cells, the amount of tyrosine-phosphorylated STAT3, but not the total amount of tyrosine-phosphorylated STAT3, was significantly reduced. In addition, the promoter activity of the *E2F*1 gene was significantly reduced if the STAT3 recognition site was deleted and if TRAP1 was knocked down. These data indicate that TRAP1 regulates the tyrosine phopsphorylation status of STAT3, which controls the expression of E2F1, and thus modulates the transcription of N-cadherin.

5.6 Role of the TRAP1-N-cadherin cascade in psychiatric disorders (Fig. 4G)

Because N-cadherin is involved in the morphogenesis of synapses (Okamura et al., 2004; Togashi et al., 2002), the regulation of the morphology of dendritic spines by TRAP1 via N-cadherin was analyzed in cultured hippocampal neurons. Spines are divided into 2 types based on morphology, namely: pedunculated and sessile with the former possessing a substantial stalk construction that is absent in the latter (Greg et al., 1999). In TRAP1 knockdown neurons, spines were predominantly sessile. Only 20.8% of spines displayed a pedunculate morphology compared with 66.7% in control neurons. Functionally, N-cadherin regulates synaptic plasticity; The activity dependent accumulation of N-cadherin

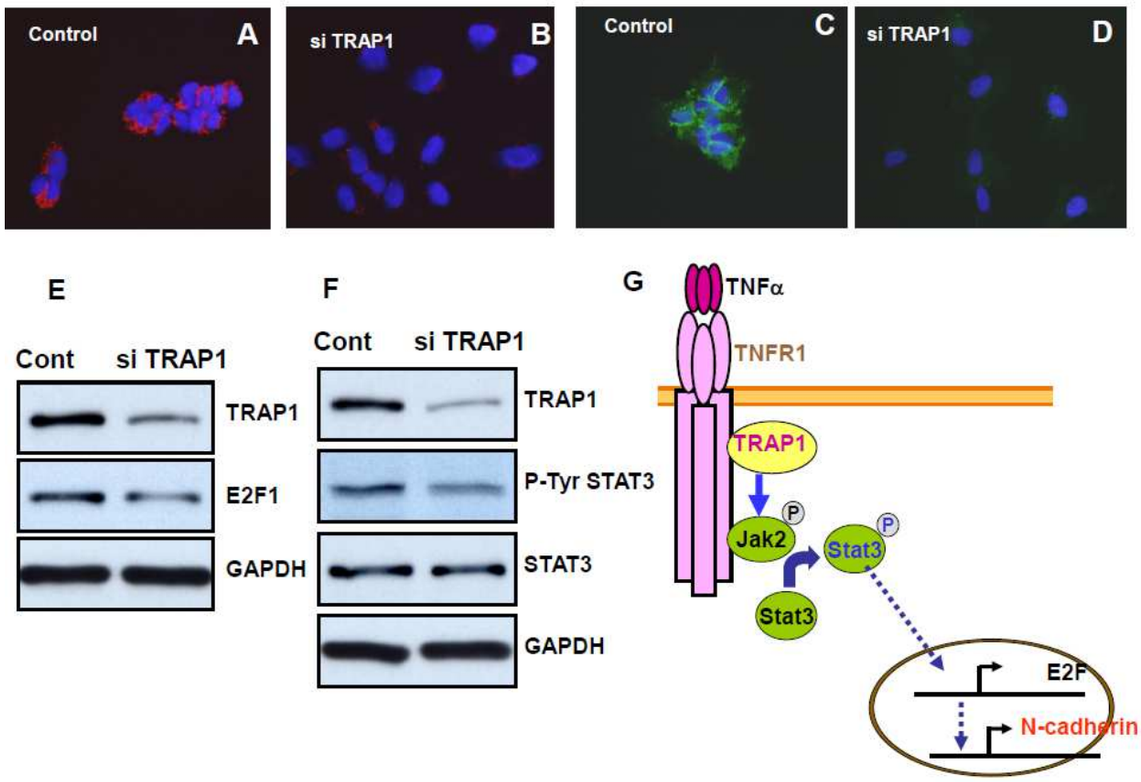


Fig. 4. TRAP1 is involved in cell-cell adhesion. A,B: Immunohistochemistry of TRAP1 knockdown and control cells stained with an anti-TRAP1 antibody (red) and DAPI(blue). TRAP1 protein levels are markedly reduced in B. In addition, cells transfected with siTRAP1 were dispersed throughout the dish (B), compared withcells transfected with control siRNAthat grew in aggregates resembling untransfected SH-SY5Y cells. C,D: TRAP1 knockdown results in the down-regulation of N-cadherin. Immunohistochemistry of TRAP1 knockdown cells (D) compared with that of control cells (C) stained with an anti-N-cadherin antibody (green) and DAPI (blue). In the TRAP1 knockdown cells (D), N-cadherin is remarkably decreased throughout the cytoplasm, including around the membrane where cell-adhesion takes place. C: Immunobloting of TRAP1 knockdown cells at 48 h after transfection with anti-TRAP1, E2F1 and GAPDH antibodies.E2F1 expression was significantly decreased in the TRAP1 knockdown cells, showing that TRAP1 knockdown decreases the transcription activity of the N-cadherin promoter. D: Immunoblotting of TRAP1 knockdown cells with anti-TRAP1, phosphorylated STAT3 (Tyr705) (p-Tyr STAT3), STAT3 and GAPDH antibodies 48h after transfection. The level of tyrosine-phosphorylated STAT was significantly reduced in the TRAP1 knockdown cells. D: Molecular pathway of the TRAP1-N-cadherin cascade. TRAP1 regulates the tyrosine phosphorylation of STAT3, which controls the expression of E2F, and thus, subsequently modulates the transcription of N-cadherin. As TRAP1 mutations are deeply involved in major depression, the disturbance of cell adhesion by the reduction of N-cadherin which causes abnormal neural circuit formation, may be important in the pathogenesis of major depression.

at synapse is essential for spine remodeling and long term potentiation, suggesting that N-cadherin plays important roles in higher brain function such as learning and memory. It is

therefore likely that altered expression of N-cadherin may be associated with the pathogenesis of mental disorders.

In support of this, we showed that 4 SNPs in the TRAP1 gene may be associated with the pathogenesis of mental disorders, particularly major depression, including 2 SNPs that cause an amino acid change in the TRAP1 protein:R07G (rs1 3926) and D395E (rs1 136948). Moreover, these 2 non-synonymous SNPs are located in the region critical for the binding of TRAP1 to TNFR1, suggesting that the binding affinity of TRAP1 to TNFR1 or the downstream signaling of TRAP1 might be altered in these disorders.

6. Involvement of DISC1 in psychiatric disorders (Fig. 5)

DISC1 has been identified as a potential susceptibility gene for major psychiatric disorders. Disruption of this gene by a balanced translocation (1:11,q42.1;q14.3) results in a predicted C-terminal truncation of the open reading frame. Furthermore, this anomaly is segregated with schizophrenia and affective disorders in a large Scottish family (Millar et al., 2000,2001). A frameshift mutation of DISC1 has been identified in an American family with schizophrenia and schizoaffective disorder (Sach et al., 2005), and the association of the SNPs of DISC1 with schizophrenia, schizoaffetive disorder and bipolar disorder has also been suggested (Hodgkinson et al., 2004).

6.1 Interacting partners bind to the area close to the translocation breakpoint of DISC1

DISC1 has been proposed to be a multifunctional protein that interacts with multifunctional protein that interact with multiple proteins of the centrosome and cytoskeletal system at a distinct domain (Morris et al., 2003; Ozaki et al., 2003). The function of DISC1 could therefore be regulated by DISC1 binding proteins. The DISC1 binding partners, fasciculation and elongation protein zeta-1 (Fez1), DBZ and kendrin were identified using yeast 2-hybrid analysis. The interaction between DISC1 and Fez1, DISC1 and DBZ, and DISC1 and Kendrin were confirmed by immunoprecipitation assays（Matsuzaki & Tohyama, 2007, Miyoshi et al., 2003, Hattori et al., 2007）.

6.2 Role of DISC1-Fez1 interaction in psychiatric disorders (Miyoshi et al.,2003)

The DISC1-Fez1 interaction identified *in vitro* was confirmed *in vivo* by showing the co-localization of DISC1 and Fez1 in neurons of the hippocampus, cerebral cortex and olfactory bulb. Analysis of the intracellular localization of DISC1 revealed that DISC1 and Fez1 co-localize in growth cones in cultured hippocampal neurons. The interactions of these proteins are associated with F-actin. The finding that a molecular complex composed of DISC1, Fez1 and actin is located in the growth cone of neurite suggests the involvement of the DISC1-Fez1 interaction in neurite extension. In support of this, both DISC1 and Fez1 were found to be expressed in the brain during an early ontogenetical stage Honda et al., 2004). The physiological role of the DISC1-Fez1 interaction in neuronal cells, especially at the stage of neurite outgrowth, was examined using PC12 cells. After stimulation with nerve growth factor (NGF), PC12 cells cease proliferation and begin to extend neuritis. The interaction between FLAG-tagged DISC1 and endogenous Fez1 was examined over the course of neuronal differentiation. The amount of Fez1 in immunoprecipitates obtained using an anti-FLAG antibody was drastically increased upon NGF stimulation, suggesting that the DISC1-

Fez1 interaction is up-regulated by NGF stimulation.. Furthermore, when treated with NGF, DISC1-stable lines exhibited enhanced neurite extension compared to mock-stable cells. These findings established that DISC1 participates in neurite outgrowth through its interaction with Fez1. In schizophrenia with DISC1 translocation carriers, in which Fez1 cannot bind to DISC1 owing to its translocation, neuronal circuit formation may remain immature. In addition, an association between the SNPs of the *Fez1* gene and schizophrenia has also been suggested in a Japanese population (Yamada et al., 2004).

6.3 Role of the DISC1-Kendrin interaction in psychiatric disorders (Miyoshi et al., 2004; Shimizu et al. 2008)

6.3.1 The carboxy-terminal region of DISC1 is essential for the DISC1-Kendrin interaction

Kendrin, also referred to as pericentrin-B, is a calmodulin-binding protein localized specifically on centrosomes. Through the presence of the PACT domain, Kendrin is targeted to the centrosome. Co-localization of DISC1 and Kendrin was demonstrated in SH-SY5Y neuroblastoma cells transfected. In addition, co-localization of DISC1 and Kendrin at the centrosome was confirmed by immunohisotchemistry. DISC1 lacking the putative Kendrin binding region (amino acid 446-553) (KBR) is unable to target to the centrosome and distributes diffusely throughout the cytoplasm, showing that interaction of DISC1 with Kendrinis essential for its centrosomal localization. A direct yeast 2-hybrid interaction assay suggested that a short fragment of amino acids 446-533 of DISC1 constituting the binding region (KBR) was essential for the interaction with Kendrin. A subsequent study using immunoprecipitation assays in HEK293 cells in which Kendrin was endogenously expressed confirmed that KBR is critical for the interaction with Kendrin as described below. Cells were transiently transfected with expression vectors for HA-tagged full-length DISC1 (DISC1-HA) and the HA-tagged DISC1 deletion muctant lacking the KBR (DSC1⊿KBR-HA). Endogenous Kendrin was coimmunoprecipitated with DISC1-HA, but not with DISC1⊿KBR-HA. These findings indicate that KBR is the binding region of DISC1 to Kendrin. To examine whether KBR itself could bind to Kendrin, several DISC1 deletion mutants were prepared: GDBP (amino acids 348-597—FLAG, BPC (amino acids 598-854)-FLAG, BR (amino acids 446-633)-FLAG, KBR-FLAG and KBRC (amino acids 446-854)-FLAG. Surprisingly, endogenous Kendrin was detected in immunoprecipitates from cells transfected with KBRC-FLAG, but it was barely detected in immunoprecipitates from cells transfected with GDBP-FLAG, BPC-FLAG, BR-FRAG or KBR-FLAG. These findings confirm KBR as the Kendrin binding region for DISC1 to Kendrin, but also show that the binding to Kendrin is enhanced remarkably in the presence of the carboxy-terminal region downstream of KBR. Thus, KBR is required but not sufficient for the interaction, and the carboxy-terminal region of DISC1 is also indispensable for the binding to Kendrin.

6.3.2 The carboxy-terminal region of DISC1 is required for the localization of DISC1 to the centrosome

Next, we determined which part of DISC1 is indispensable for co-localization of DISC1 with Kendrin at the centrosome. KBRC-FLAG showed a diffuse pattern in the cytoplasm but clearly revealed a strong ‘dot' pattern in the perinuclear area. The merged image of KBRC-FLAG and Kendrin showed that they were colocalized at the centrosome. On the other hand, localization of KBR-FLAG showed a diffuse distribution pattern in the nucleus and

cytoplasm without strong staining at the centrosome. Staining of BR-FLAG and GDBP-FLAG was characterized by a small punctate distribution pattern, while BPC-FLAG exhibited a diffuse distribution in the cytoplasm. However, BPC-FLAG was not detected at the centrosome. Taken together, these findings demonstrate that the carboxy-terminal half of the DISC1 protein containing KBR and the downstream region of KBR are necessary and sufficient to target the DISC1 protein to the centrosome.

6.3.4 Inhibition of the DISC1-Kendrin interaction perturbs the microtubule network formation

The interaction of DISC1 with Kendrin at the centrosome and the key role of Kendrin in microtubule nucleation at the centrosome suggest that the interaction between DISC1 and Kendrin may affect microtubule network formation. The DISC1-binding region of Kendrin (DBR) (amino acids 2918-305) was first identified. To inhibit the DISC1-Kendrin interaction specifically at the centrosome, a FLAG-tagged DRB-PACT (DBR-PACT-FLAG) construct was prepared including DBR and PACT (a conserved centrosomal targeting motif in CG-NAP and pericentrin). Microtubule aster formation was then observed in COS cells transfected with either the DBZ-PACT-FLAG or PACT-FLAG expression vector. Mock-transfected cells showed microtubule aster formation at the centrosome. However, over-expression of DBR-PACT-FLAG resulted in a significant decrease in the percentages of cells containing the microtubule aster compared with cells expressing PACT-FLAG. In addition, over-expression of DISC1 ⊿KBR-FLAG resulted in a significant decrease in the percentage of cells containing the microtubule aster compared with mock-transfected cells. These results show that the DISC1-Kendrin interaction is involved in brain maturation through the regulation of microtubule organization.

6.3.5 Role of the DISC1-Kendrin interaction in mental disorders

Over-expression of the DISC1-binding region of Kendrin perturbed the normal distribution of the stabilized microtubule network. The over-expression of DISC1 lacking the Kendrin binding site caused an impairment in microtubule aster formation. Carriers of the chromosomal translocation that segregates with mental diseases are expected to produce the truncated mutant DISC1 protein that lacks the carboxy-terminal region, or to have a reduced expression of the DISC1 protein. In the case of truncated mutant protein expression, this protein would not be able to target to the centrosome and interact with Kendrin, which might induce dysfunction of the microtubule network formation. Loss of DISC1 protein expression could lead to the dysfunction of microtubules by disrupting the DISC1-Kendrin interaction. In addition, involvement of Kendrin in olfactory cilia assembly was also reported (Miyoshi et al., 2009). Thus, the DISC1-Kendrin interaction plays a role in neuronal development by regulating microtubule organization, showing that mental diseases derived from DISC1 dysfunction are neurodevelopmental disease. In addition, our recent analysis showed an association between SNPs of the Kendrin gene and bipolar diseases (Anitha et al., 2005).

6.4 Role of DISC1 and its binding proteins with special reference to psychiatric disorders

The present review summarize results showing that DISC1 functions in neural network formation by interacting with several binding partners, including Fez1, DBZ, and

Kendrin, which binds to the area near the translocation site of DISC1. DISC1 interactions, including DISC1-Fez1, DISC1-DBZ and DISC1-Kendrin, all play a key role in neuronal development. Other groups have identified additional DISC1 interaction partner such as NudE-like (NUDEL) (Morris et al., 200; Ozeki et al., 2003), lissenchphaly-1 (LIS1) (Brandron et al., 2004), phosphodiesterase 4B (PDE4B) (Millar et al., 2005), glycogen synthase kinase 3 (GSK3) (Mao et al., 2009), the motor protein dynein and growth factor receptor bound protein 2 (Grb2) (Shinoda et al., 2007;Taya et al., 2007), and these binding partners suggested the functional involvement of DISC1 in neural development. DISC1 therefore plays crucial roles in brain development by affecting neuronal migration, neurite outgrowth and neural maturation through its interaction with several cytoskeletal proteins.

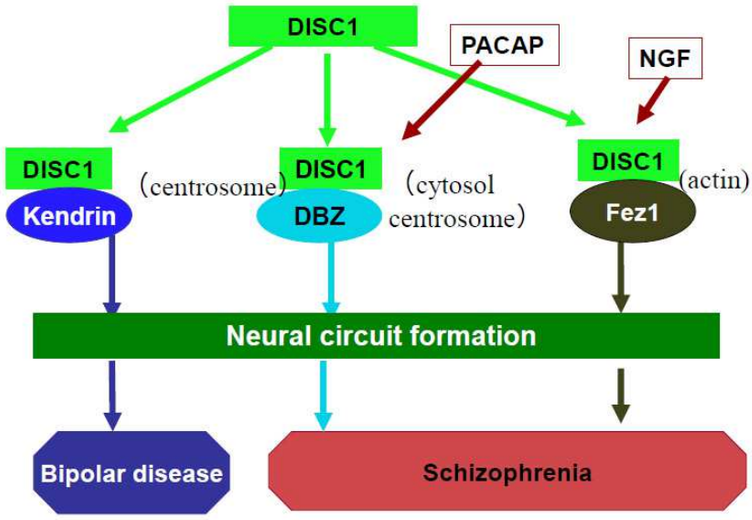


Fig. 5. DISC1 binding proteins that bind to an area near the translocation and their function. The binding between DISC1 and these molecules play a role in neurodevelopment. Accordingly mental disorders in which these molecules are implicated could be considered neurodevelopmental diseases.

7. Conclusion

The results summarized in this review indicate that the TRAP1 cascade, PACAP-stathmin1 cascade, PACAP-DBZ/DISC1 cascade, Dysbindin-MARCKS cascade, DISC1-Fez1 interaction and DISC1-Kendrin interaction are all involved in neural development. In addition, the molecules mentioned above are associated with schizophrenia or bipolar disease, showing that the neural development that is associated with these systems is disturbed in the brains of patients with either of these disease.

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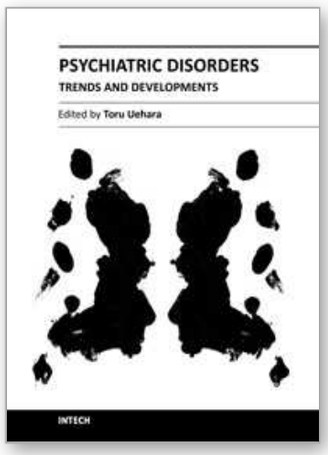
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Due to their prevalence, pervasiveness and burden inflicted on men and women of today, psychiatric disorders are considered as one of the most important, sever and painful illnesses. This impairment of cognitive, emotional, or behavioural functioning is in some cases tragic. Aside from knowing the physical organic factors, such as infections, endocrinal illnesses or head injuries, the aetiology of psychiatric disorders has remained a mystery. However, recent advances in psychiatry and neuroscience have been successful in discovering subsequent pathophysiology and reaching associated bio-psycho-social factors. This book consists of recent trends and developments in psychiatry from all over the world, presented in the form of multifarious and comprehensive articles. The first two sections of the book are reserved for articles on schizophrenia and depression, two major illnesses present in this field. The third section of the book is reserved for addiction psychiatry, related not only to socio-cultural but also biological alterations. The last section of the book, titled Biological Neuropsychiatry, consists of three topics - updated molecular biology, fundamental neuroscience and clinical neuropsychiatric conditions. Doubtlessly, this book will be fruitful for future developments and collaboration in world psychiatry.

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