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FEATURE REVIEW

The *DISC* locus in psychiatric illness

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The *DISC* locus is located at the breakpoint of a balanced t(1;11) chromosomal translocation in a large and unique Scottish family. This translocation segregates in a highly statistically significant manner with a broad diagnosis of psychiatric illness, including schizophrenia, bipolar disorder and major depression, as well as with a narrow diagnosis of schizophrenia alone. Two novel genes were identified at this locus and due to the high prevalence of schizophrenia in this family, they were named *Disrupted-in-Schizophrenia-1 (DISC1)* and *Disrupted-in-Schizophrenia-2 (DISC2)*. *DISC1* encodes a novel multifunctional scaffold protein, whereas *DISC2* is a putative noncoding RNA gene antisense to *DISC1*. A number of independent genetic linkage and association studies in diverse populations support the original linkage findings in the Scottish family and genetic evidence now implicates the *DISC* locus in susceptibility to schizophrenia, schizoaffective disorder, bipolar disorder and major depression as well as various cognitive traits. Despite this, with the exception of the t(1;11) translocation, robust evidence for a functional variant(s) is still lacking and genetic heterogeneity is likely. Of the two genes identified at this locus, *DISC1* has been prioritized as the most probable candidate susceptibility gene for psychiatric illness, as its protein sequence is directly disrupted by the translocation. Much research has been undertaken in recent years to elucidate the biological functions of the DISC1 protein and to further our understanding of how it contributes to the pathogenesis of schizophrenia. These data are the main subject of this review; however, the potential involvement of *DISC2* in the pathogenesis of psychiatric illness is also discussed. A detailed picture of DISC1 function is now emerging, which encompasses roles in neurodevelopment, cytoskeletal function and cAMP signalling, and several DISC1 interactors have also been defined as independent genetic susceptibility factors for psychiatric illness. DISC1 is a hub protein in a multidimensional risk pathway for major mental illness, and studies of this pathway are opening up opportunities for a better understanding of causality and possible mechanisms of intervention.
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Genetics of *DISC1* discovery

St Clair *et al.*[1] first reported a Scottish family with a high loading of major mental illness, which co-segregates with a t(1;11) translocation. Long-term follow-up of this family over a period of 30 years has reported 87 family members, of whom 37 carry the translocation.[2] Of 29 individuals carrying the translocation, for whom psychiatric assessment was possible, 7 have a diagnosis of schizophrenia, 1 has a diagnosis of bipolar disorder and 10 cases of recurrent major depression were also reported.[2] Thus, 18 of 29 translocation carriers are diagnosed with major mental illness whereas none of 38 non-translocation carriers have such a diagnosis, providing strong evidence for a causal link between the t(1;11) and the psychiatric liability in this unique family. This translocation segregates in a highly statistically significant manner with the wide spectrum of psychiatric illnesses in this family (log of the odds ratio (LOD) = 7.1 for the broad diagnosis). Linkage with schizophrenia alone is also significant, producing an LOD score of 3.6.[2] Blackwood *et al.*[2,3] further reported that the psychiatric presentations are typical with no other distinguishing clinical features. They also reported that, in unaffected translocation carriers, the latency and amplitude of the event related potential (ERP) P300, a measure of the speed and efficiency of information processing, is indistinguishable from that of affected individuals. Moreover, as a group the translocation carriers show the characteristic abnormal P300 ERP associated with schizophrenia and bipolar disorder.[2]

The pattern of inheritance in the t(1;11) family is thus consistent with a simple dominant mode of inheritance with reduced penetrance, with altered P300 as a correlated endophenotype. We can speculate that secondary and independently segregating

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genetic risk factors, variable environmental exposures or stochastic events may influence the presence or absence of clinical signs and the specific psychiatric diagnosis, but in this family the t(1;11) accounts for essentially all of the transmitted risk.

Major exercises were undertaken to fine-map and then clone the breakpoint. Microdissection identified the breakpoint regions on chromosomes 1 and 11.[4] A contiguous map of bacterial artificial chromosome, yeast artificial chromosome and cosmid clones was then established across the chromosome 11 region.[5] At the same time, no evidence for a gene at the breakpoint on chromosome 11 was found.[6,7] The closest known genes to the breakpoint on chromosome 11 are a transcribed α-tubulin pseudogene at 328 kb and NAALADdase II at 372 kb proximal to the breakpoint.[8] There are, however, now novel expressed sequence tags (EST) overlapping this chromosome 11 breakpoint region, which suggest that gene/s may exist in this region, albeit yet undefined. Attention turned to the chromosome 1 component of the translocation which was cloned and sequenced, revealing the *DISC1* and *DISC2* genes.[9,10]

Independent evidence from linkage

The first independent evidence for the involvement of the *DISC1* locus in psychiatric illness came from studies of the Finnish population. Two linkage studies, one based on the entire Finnish population and the other on an internal Finnish isolate, have provided evidence for linkage of schizophrenia and schizoaffective disorder to the 1q32.2–q41 region of chromosome 1, proximal to the *DISC1* gene.[11,12] Fine-mapping of this region further refined the linkage peak, with the maximum LOD score detected at a microsatellite within intron 9 of the *DISC1* gene.[13] Importantly, this linkage was replicated by Ekelund *et al*.[14] in an independent Finnish population sample for a SNP within intron 9.

Studies of populations including those of Taiwan, Scotland and Britain/Iceland have also provided evidence for linkage of chromosome 1q32–42 to major psychiatric illness.[15–19]

Independent evidence from association

Devon *et al*.[20] sequenced the exons and intron/exon boundaries of *DISC1* and reported 15 novel polymorphisms including the common Ser704Cys (rs821616) polymorphism in exon 11. No evidence of single marker association was observed, but this was a preliminary study with low coverage designed primarily to discover and report potentially useful polymorphisms.[20]

The first positive report of association came again from the Finnish population. As an extension to their linkage study, Ekelund *et al*.[14] also identified a three-SNP haplotype spanning exon 9 of *DISC1*, which is associated with a broad diagnosis of schizophrenia, schizoaffective and schizophrenia spectrum. In a subsequent follow-up study (which was actually published first), Hennah *et al*.[21] reported four *DISC1* haplotypes (HEP1-4), which are associated with the same broad diagnosis of affected status. Of these haplotypes, HEP3, which spans 62 kb from intron 1 to exon 2, was the most statistically robust.[21] This HEP3 haplotype is significantly associated with delusions, hallucinations and negative-component traits in affected females.[21] Although originally reported as being undertransmitted to affected females,[21] it was later reported that in fact this haplotype is overtransmitted to affected males in this population.[22,23]

Association of a haplotype overlapping HEP3 (called Hap 1) has further confirmed this finding in an independent study of North American schizoaffective patients.[24] However, in this population, the Hap 1 haplotype is reported to be undertransmitted to the affected individuals. This North American study also provided evidence for association of additional *DISC1* haplotypes (spanning exon 1–exon 9) with schizophrenia, schizoaffective and bipolar disorder, and a single marker association with a nonsynonymous Leu607Phe amino-acid substitution (a SNP within the Finnish HEP1 haplotype).[24] A second independent North American study by Callicott *et al*.[25] reported positive association for a three-SNP haplotype of *DISC1* spanning 83 kb from intron 9–exon 11, which contains the nonsynonymous serine to cysteine amino-acid substitution at position 704. This Ser704Cys SNP has also recently been associated with schizophrenia in a Han-Chinese population[26] and with risk for developing major depression in a Japanese population.[27]

Thomson *et al*.[28] reported the first comprehensive linkage disequilibrium map of the *DISC* locus and performed an association study using tagging SNPs in a Scottish population sample diagnosed with schizophrenia and bipolar disorder. Consistent with the linkage results from this population,[17] the strongest association was seen with bipolar disorder (four-SNP haplotype spanning intron 4–intron 6 of *DISC1*), although some evidence for association was also seen for schizophrenia (two-SNP haplotype within intron 6 of *DISC1*).[28] The association with bipolar disorder, like that seen for schizophrenia in the Finnish population, is sex specific, with the most statistically robust association detected with this four-SNP haplotype and bipolar disorder in female subjects.[28] Additional sex-specific associations for *DISC1* have been reported within the Han-Chinese and Japanese populations, making this a common finding for the *DISC* locus in relation to its involvement in psychiatric illness.[27,29]

A recent association study of Finnish autism and Asperger families detected sex-specific associations of *DISC1* with autism spectrum disorders and Asperger syndrome in affected male subjects.[30] This study implicated a microsatellite within *DISC1* intron 9, and a three-SNP haplotype, encompassing HEP3, both of which have previously displayed linkage or

association to schizophrenia in the Finnish population. These data re-enforce the idea that many neuropsychiatric disorders involving cognitive deficits may have overlapping genetic susceptibility factors and that *DISC1* is strongly implicated as one of these.

As with any of the current candidate genes for psychiatric illness there have also been negative reports of association;[20,31,32] however, the accumulation of positive genetic evidence for involvement of the *DISC* locus in conferring predisposition to psychiatric illness is encouraging. Comparisons between the association studies have unfortunately been hampered due to substantial differences in the coverage of the *DISC* locus and the specific markers used. Table 1a provides a summary of the genetic findings discussed in this section, including the details of the specific markers tested in each of the above studies.

With the exception of the t(1;11) translocation, no definitive causal variants have yet been identified in psychiatric patients. Sachs *et al*.[33] recently reported a 4-bp frameshift mutation at the 3′ end of exon 12 of *DISC1* in two siblings with schizophrenia, and one with schizoaffective disorder, from a North American pedigree. This mutation was also identified in the asymptomatic father of these siblings but not in one sibling with schizotypal personality disorder or two siblings diagnosed with major depression. Since this original study, the mutation has been studied in a British/Irish cohort, where it was not identified in schizophrenia subjects but was detected in two control subjects.[34] These controls were however anonymous blood donors that did not undergo psychiatric assessment. Therefore, the relationship that this mutation bears to risk for psychiatric illness remains to be determined.

DISC1 variation and cognitive abilities

One of the most consistent observations in schizophrenic individuals, and to a lesser extent in bipolar individuals, is a specific impairment of cognitive ability. The first indication that *DISC1* variation might have subclinical effects came from the demonstration of altered ERP P300 in unaffected t(1;11) carriers.[2] A number of studies have since reported evidence of linkage or association between the *DISC1* locus and impaired cognitive function in both schizophrenic and normal individuals (Table 1b, Figure 1a).[22,25,35–40] These deficits are consistent with dysfunction in the dorsolateral prefrontal cortex (PFC) and the hippocampus.

Gasperoni *et al*.[37] did not test *DISC1* directly, but did find evidence of linkage to spatial working memory function in the *DISC1* region of chromosome 1q42.4 in the Finnish population. In an extension of their association studies on Finnish schizophrenia cases, Hennah *et al*.[22] reported association for HEP3 to visual working memory and Paunio *et al*.[40] found evidence for linkage of the 1q32–1q42 region to verbal learning and semantic clustering.

Burdick *et al*.[35] reported that two *DISC1* SNPs, within introns 6 and 8, are associated with rapid visual search and working memory. As a cautionary note, these associations were significant only in their African-American sample and not within the European/American sample, inconsistent with the Finnish/American studies.[35] More recently, a two-SNP haplotype spanning introns 4 and 5 of *DISC1* was also shown to be associated with deficits in sustained attention in the Taiwanese population.[39]

A number of studies have reported cognitive deficits associated with the common Ser704Cys *DISC1* polymorphism. Callicott *et al*.[25] showed association of the Ser704Cys allele with schizophrenia, and the serine allele of this SNP was further shown to be associated with cognitive defects in both schizophrenic and normal subjects. In schizophrenia subjects, homozygosity of the Ser704 allele is associated with reduced levels of *N*-acetyl-aspartate and reduced performance on the Wechsler memory scale.[25] This Ser704 allele is also associated with reduced hippocampal grey matter volume and abnormal activation of the hippocampus during the N-back working memory task in healthy individuals, and with reduced performance on the Wisconsin card sorting test in both schizophrenia and controls subjects.[25] A similar trend towards decreased hippocampal volume has also been reported in the Finnish population for the *DISC1*-associated haplotype HEP1.[36] In contrast to these studies, Hashimoto *et al*.[27] did not find decreases in hippocampal volume associated with the Ser704Cys polymorphism in their Japanese control sample; however, they did report decreases in the grey matter of the cingulate cortex, cingulate gyrus and the posterior gyrus, and abnormalities in prefrontal white matter, in carriers of the Cys704 allele. Therefore, while this study supports the idea that the Ser704Cys polymorphism is associated with reductions in brain tissue volume, the regions of volume reduction differ and the allele associated with the reductions differs also.

Thomson *et al*.[38] further examined the effect of the Ser704Cys variant on cognitive ability in a unique Scottish cohort. No correlation was found for any of the cognitive domains tested. However, these individuals were part of the Scottish Mental Survey of 1937 who had been tested at age 11 for general cognitive ability by the Moray House Test. As this test had been repeated again at age 79, it was possible to test for an effect on cognitive aging. Females homozygous for the Cys allele showed a poorer aging profile than males.[38]

Summary of genetic data

The *DISC* locus has now been implicated by cytogenetics, linkage and association as a predisposing risk factor for neuropsychiatric illness, including schizophrenia, schizophrenia spectrum, bipolar, depression and autism spectrum disorders. Despite a few negative reports, the accumulating genetic evidence is strongly positive. However, the major hurdle that

Table 1 *DISC1* linkage and association findings

(a) DISC1 linkage and association findings

Study no.	Sample phenotype	Marker/haplotype	Location relative to DISC1	Population
Linkage and family studies				
19	BP	D1S1660-D1S1678 (1q32)	28 Mb proximal	North America
12	SCZ/SCZaff	D1S2891	23 Mb proximal	Finnish Isolate
11	SCZ/SS/MD/BP	D1S2833	1 Mb proximal	Finland
2	SCZ/BP/MD	T(1;11)	Intron 8	Scottish Family
13	SCZ/SCZaff/SS	D1S2709	Intron 9	Finland
18	BP/UP	D1S251	48 kb proximal	Britain/Iceland
16	SCZ/SCZaff/NApsy	D1S251	48 kb proximal	Taiwan
14	SCZ/SCZaff/SS	rs1000731	Intron 9	Finland
17	BP	D1S103	1 Mb proximal	Scotland
15	SCZaff	Microsatellite at 1q42.2	2.5 Mb distal	Britain/Ireland
33	SCZ/SCZaff	Frameshift mutation	Exon 12	N.American family
Association studies				
21	SCZ/SCZaff/SS/BP/MD	HEP1	Exon 9–intron 9	Finland
		HEP2*	Intron 4 (TRAX)	
		HEP3 (sex-specific)	Intron1-Exon2	
		HEP4*	Exon 13	
14	SCZ/SCZaff/SS	HEP1	Exon 9–intron 9	Finland
24	SCZaff	rs6675281 (Leu607Phe)	Exon 9	North America
		Haploblock 3 (hap3 and hap8)	Intron 8–intron 9	
25	SCZ	rs821616 (Ser704Cys)	Exon 11	North America
		3 SNP haplotype	Intron 9–exon 11	
36	SCZ	HEP1	Exon 9–intron 9	Finland
		HEP2/3	TRAX/DISC1	
28	BP	SNPs 10–13	Intron 4–intron 6	Scotland
	SCZ	SNPs 12–13*	Intron 6	
27	MD	Ser704Cys (rs821616)	Exon 11	Japan
	SCZ	rs821577 (sex-specific)	Intron 9	
85	BP	12 SNP haplotype*	Intron 1–exon 13	American
39	SCZ	rs2793092-rs2793091 (haploblock 2)	Intron 3–intron 4	Taiwan
29	SCZ	rs2295959 (sex-specific)*	Intron 9	Han Chinese
26	SCZ	rs821616 (Ser704Cys)	Exon 11	Han Chinese
		rs821597	Intron 10	
		rs821597 + rs4658971 + rs843979 + rs821616	Intron 10–exon 11	
30	ASD	D1S2709 (sex-specific)	Intron 9	Finland
	AS	rs1322784 (sex-specific)	Intron 6	
	AS	HEP3 + rs1322784 (sex-specific)	Intron 1–intron 6	

(b) DISC1 variation affecting brain structure and cognition

Study no.	Genetic variant	Phenotype	Population
2	T(1;11)	Abnormal P300 event-related potential	Scottish family
35	HCV12001930 (rs2255340) and hCV1650649	Rapid visual search and working memory	African-American
25	Ser704 (rs821616)	Reduced hippocampal grey matter volume	North America
		Reduced NAA levels	
		Abnormal hippocampal activation	
36	HEP1 and HEP2/3	Reduced PFC grey matter	Finland
	HEP1	Impaired long-term memory	
	HEP2/3	Impaired spatial working memory	
22	HEP3	Visual working memory	Finland

Table 1 Continued

(b) DISC1 variation affecting brain structure and cognition			
Study no.	*Genetic variant*	*Phenotype*	*Population*
38	Cys704 (rs821616)	Abnormal cognitive ageing in females	Scotland
27	Cys704 (rs821616)	Reduced grey matter volume in cingulate cortex, cingulate gyrus and posterior gyrus Reduced white matter integrity in PFC	Japan
39	rs2793092-rs2793091	Sustained attention deficits	Taiwan

AS, Asperger syndrome; ASD, autism spectrum disorders; BP, bipolar disorder; MD, major depression; NApsy, non-affective psychosis; SCZ, schizophrenia; SCZaff; schizoaffective disorder; SS, schizophrenia spectrum.
Part (a) depicts the location of markers and haplotypes, which display positive linkage and association with psychiatric illness. Earlier linkage studies have implicated the 1q region harbouring the *DISC* locus, and this has been refined by association studies of the *DISC* genes. A wide range of markers and haplotypes display association, which together span the full length of the *DISC* locus. For studies with multiple linkage/association findings, the most robust findings are reported here. Less robust findings are marked with an asterisk. The location of markers and haplotypes are listed according to the Mar 2006 version of the UCSC human genome browser. Part (b) shows the associations of *DISC1* variants to various cognitive deficits. These deficits are representative of cognitive endophenotypes of schizophrenia and other major psychiatric illnesses.

we must now overcome is to understand what the genetic data are telling us. A number of nonsynonymous changes have been reported in the *DISC1* gene, although their impact on DISC1 protein expression or function is not yet clear. It is clear, however, that no specific allele or variant or even gene region is consistently associated with the risk of developing psychiatric illness (Table 1a). This suggests that there is allelic heterogeneity for *DISC1* in psychiatric illness, and also implies that associated variants are not necessarily causative, but may be in linkage disequilibrium with as yet unidentified risk variants. In short, the genetic data tell us that the search must go on. We need to focus now on finding and characterizing the causative variants that may alter the functionality of the *DISC* locus. While doing this we must be aware that a functional variant does not necessarily need to change the amino-acid sequence of the protein, as changes at the nucleotide level may affect regulation of gene expression through transcriptional, processing or translational mechanisms. In this regard, a precedent has been set recently with the report of 'silent' mutations in the *MDR1* gene that do not change the amino-acid sequence, but are likely to affect translation and cotranslational protein folding, which alters the conformation and activity of the resulting P-glycoprotein.[41] For a protein such as DISC1, which binds a number of interacting proteins, secondary structure will undoubtedly be important, therefore 'silent' mutations that may affect protein translation or folding may alter its ability to perform interactions.

The phenotype of mouse Disc1 variants

Modelling human psychiatric illness in animals is a controversial issue, as many aspects of mental disorders, such as hallucinations and delusions, are likely to be human specific. However, a number of anatomical, cognitive and behavioural characteristics have been identified in psychiatric patients, and it is possible to look for such deficits in animals to test the validity of proposed models of mental illness (for a review, see Arguello and Gogos[42]).

Identification of genetic risk factors for mental illness, such as *DISC1*, will facilitate the process of generating animal models and reveals a great deal about molecular mechanisms that may be deficient in human patients. Although many studies indicate DISC1 involvement in major psychiatric illness, the t(1;11) translocation is the only causal variant that has been definitively identified so far.[9] It has been speculated that a C-terminally truncated form of DISC1 encoded by exons 1–8 may be expressed from the translocated allele, and indeed transcription of *DISC1* has been detected from the derived chromosome 1 in lymphoblastoid cell lines derived from translocation carriers.[43] However, overall *DISC1* transcript levels are reduced, indicating that the truncated transcripts may be unstable.[43] Consistent with this, DISC1 protein expression is also reduced in lymphoblastoid cell lines carrying the translocation.[43] Importantly, an antibody that detects artificially expressed C-terminally truncated DISC1 fails to detect any such endogenous truncated protein in these lymphoblastoid cell lines, indicating that haploinsufficiency is the most likely disease mechanism in translocation carriers,[43] although it has not been possible to confirm this using brain tissue. However, in the unlikely event that a brain-specific C-terminally truncated protein is produced from the translocation, it is far from certain that it would be perfectly encoded by the remaining exons 1–8.

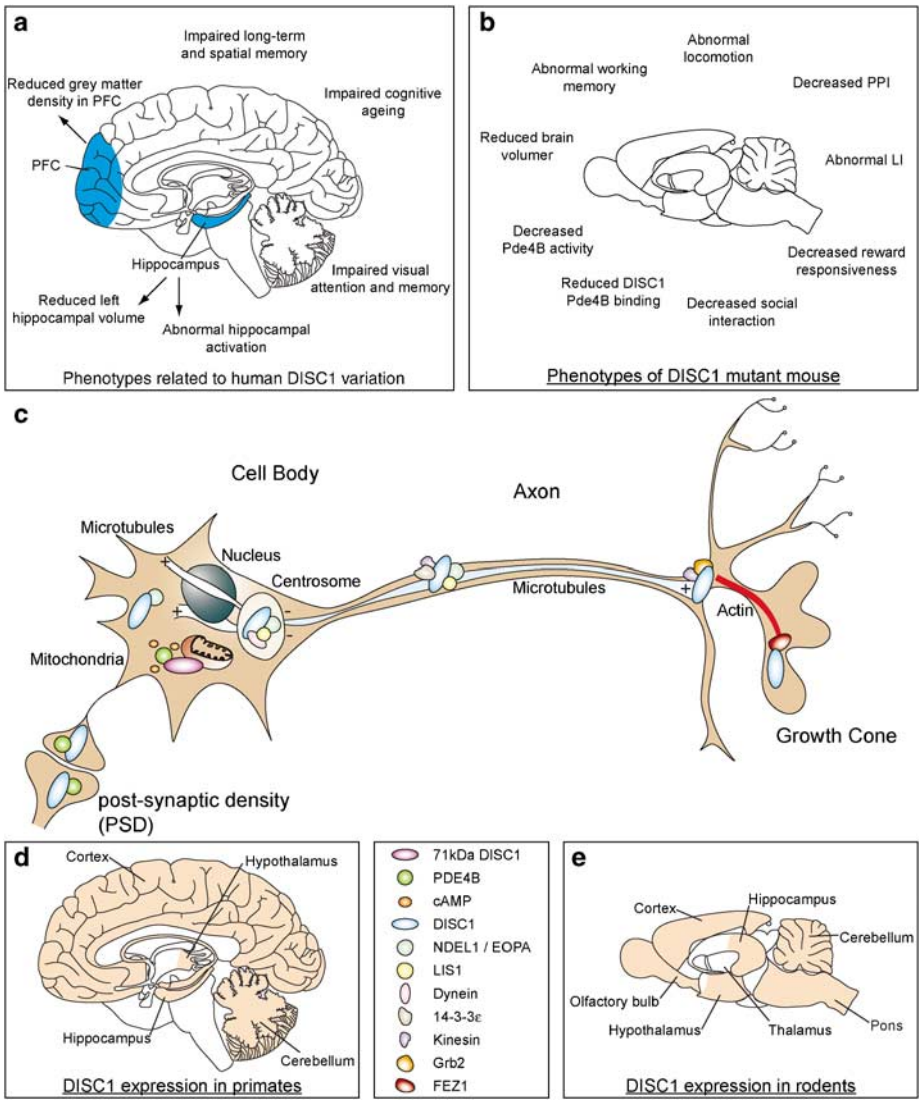

Figure 1 *DISC1* expression, cognitive and behavioural phenotypes. This figure depicts defects in a number of behavioural and cognitive domains that have been reported to be associated with genetic variants of *DISC1* in humans and mice (**a**, **b**), and are representative of reported endophenotypes of major psychiatric illness. These defects are likely to be due to the widespread expression of *DISC1* in many brain regions. This figure depicts regional *DISC1* expression at the macroscopic level in both primate and rodent brains (**d**, **e**). *DISC1* exhibits widespread expression including the hippocampus, frontal cortex and cerebellum. At the subcellular level, DISC1 expression is found in many locations including the centrosome, mitochondria and growth cones of outgrowing neurites, where it is known to interact with a number of proteins important for neuronal migration, axon elongation and cAMP signalling (**c**). DISC, Disrupted-in-Schizophrenia.

Several groups have attempted to mimic DISC1 haploinsufficiency by knocking out the mouse *Disc1* gene by conventional homologous recombination, or to generate transgenic lines overexpressing human *DISC1* transgenes of various designs, including truncated versions designed to express the hypothetical truncated protein product of the translocation. These include constitutive/selective knockout or overexpression during development or in specific cell lineages, but to date these studies are only reported in abstract form;[44] full, published studies are awaited with great interest.

For reasons that are not clear, the mouse *Disc1* locus has proved recalcitrant to conventional knockout in embryonic stem cells derived from the standard 129Sv strain. The explanation may derive at least in part from the fact that the 129Sv strain and indeed all related 129 strains carry a 25 bp frameshift deletion within exon 6 of *Disc1* resulting in a premature termination codon at exon 7.[45,46] It is worth noting that by virtue of the extensive inbreeding that took place in the past to create the 'fancy mice' that now constitute many of the commonly used laboratory mouse strains, they will inevitably carry a significant

number of strain specific homozygous mutations, including a number of behavioural variants.[47]

Koike *et al*.[45] discovered the deletion while modifying the 129 *Disc1* allele to imitate production of the hypothetical C-terminally truncated protein product of the translocation. They attempted to do so by inclusion of an artificial stop codon in exon 8, followed by an artificial polyadenylation signal. This mutated 129 allele was backcrossed onto the C57BL/6J strain, selecting at each generation for the 129-derived *Disc1* allele, and segregating away all other potentially confounding 129 genetic variants. Koike *et al*.[45] then compared the behaviour of the C57BL/6J strain to that of otherwise isogenic strains, but carrying the 129 *Disc1* variant. There are a number of commonly used tests for behavioural abnormalities and learning paradigms that are thought to correspond to aspects of schizophrenia and mood disorder (for a review, see Arguello and Gogos[42]). Kioke *et al*.[45] did not test all of these, but rather reported a significant difference in one specific test of working memory (in a delayed non-match to place test) that was significantly different from C57BL/6J in both 129 *Disc1* heterozygotes and homozygotes. The fact that heterozygotes also displayed significant differences indicates haploinsufficiency for this trait. They tested, but found no evidence for altered prepulse inhibition, a standard test for attention that is frequently abnormal in schizophrenia. They also reported a complete absence of full-length Disc1 protein in these mice, but using a novel and uncharacterized Disc1 antibody.[45] A subsequent collaborative study[48] using all available mouse *Disc1* reactive antibodies demonstrated that the 129 variant has a subtle effect, with most if not all *Disc1* isoforms expressed as normal, suggesting that the deletion is bypassed, possibly by exon skipping. This does not rule out selective and specific outcomes arising from the variant, but these remain to be demonstrated. It therefore remains to be seen whether the working memory deficits reported by Koike *et al*.[45] are due to the natural deletion in *Disc1*, the attempt to express an artificially truncated form, or a combination of both.

An attractive alternative to gene targeting is to screen for mutations in the mouse after mutagenizing with *N*-ethyl-*N*-nitrosourea.[49] In a collaboration with the RIKEN Genomic Sciences Centre (Yokohama, Japan), Clapcote *et al*.[50] screened the large exon 2 of *Disc1* and identified two different missense mutations, a Q (glutamine) to L (leucine) change at amino-acid position 31 (Q31L) or a L (leucine) to P (proline) change at position 100 (L100P). The brain anatomical, behavioural, pharmacological and biochemical phenotypes of these two *Disc1* missense mutants were also investigated[50] (Figure 1b). In brief, magnetic resonance imaging revealed that mouse brain volumes are reduced by 6% in the 31L strain and by 13% in the 100P strain, compared to wild-type mice. The volume deficits are predominantly in the cerebellum, but also in the cortex, entorhinal cortex and thalamus. These changes are consistent with the macroscopic brain abnormalities reported in schizophrenia and also, but less markedly, in bipolar affective disorder.[51] 100P mutant mice exhibit schizophrenia-like behaviour, with profound deficits in prepulse inhibition (PPI) and latent inhibition (LI), measures of sensory motor gating and attention, which are reversed by treatment with typical or atypical antipsychotics.[50] Mice with the 31L mutation also show LI deficits, but by contrast to 100P, show modest PPI deficits and a more depressive-like behaviour with a pronounced deficit (not seen in the 100P strain) in the forced swim test, a measure of despair.[50] The 31L deficit is partially reversed by the antidepressant bupropion, but not by rolipram, a phosphodiesterase 4 (PDE4) inhibitor. This study demonstrates that *Disc1* missense mutations in mice give rise to depression-like (31L) and schizophrenia-like (100P) phenotypes, thus further supporting the role of this gene in major mental illness.[50]

Two closely related studies report the effects of human *DISC1* cDNA transgenes designed to express a truncated DISC1 protein on a background of endogenous mouse Disc1 protein expression. Sawa and co-workers[52] generated transgenic lines expressing the human truncated protein controlled by the calcium-calmodulin-dependent kinase II alpha (αCaMKII) promoter, with the intention of specifying expression predominantly in forebrain neurons in neonates. Within this developmental window, transgene expression is similar to that of endogenous Disc1 in the forebrain, although endogenous Disc1 is also present at other locations throughout the brain and throughout development into adulthood (see subsequent sections). Ross and co-workers (Pletnikov *et al*.[53]) used essentially the same approach, but with an inducible expression system directed by the CaMKII promoter, and were able to detect expression from as early as embryonic day 15, suggesting that the CaMKII promoter may not be faithfully regulated.

Both groups describe brain morphological anomalies, claiming to detect increased lateral ventricle size using magnetic resonance imaging, a characteristic of many cases of schizophrenia and their unaffected relatives that is believed to be neurodevelopmental in origin. Although both groups identified increased lateral ventricle size in the absence of any change in total brain volume, Hikida *et al*.[52] detected this abnormality only at 6 weeks of age and demonstrated, rather surprisingly, that by 3 months (when the transgene is no longer expressed) there is no detectable difference in lateral ventricle size between wild-type and transgenic animals. Pletnikov *et al*.,[53] however, detected the increased lateral ventricle size in adult mice. Hikida *et al*.[52] also found evidence for loss or reversal of normal brain asymmetry in animals expressing the transgene. Pletnikov *et al*.[53] performed a detailed histological examination and detected no abnormalities of morphology, layering or cytoarchitecture within the hippocampus and cortex. The fact that there were significant differences between these

superficially similar transgenic models begs a number of questions about the mechanism that only further analysis and direct comparison will resolve.

Interestingly, when cortical neurons were isolated from embryos, they were found to exhibit significantly less complex dendritic arborization, (Pletnikov *et al*.[53]) consistent with previous studies demonstrating dominant-negative effects of truncated human DISC1 upon neurite extension and arborization (see subsequent sections). A number of behavioural tests were also carried out on the models, but unfortunately these differed between the two John Hopkins University teams so direct comparison is not possible, nor did they compare directly to the previous study of Clapcote *et al*.[50] However, in both of the truncated DISC1 transgenic studies, behavioural abnormalities were displayed that complemented those reported by Clapcote *et al*.[50] substantiating the evidence that manipulation of DISC1 induces behaviours that can be related to characteristics of human psychiatric illness. Hikida *et al*.[52] tested adult male animals only and report hyperactivity, which is believed to model psychomotor agitation, increased immobility in the forced swim test and a deficit of prepulse inhibition, but only at one of the frequency tested. In this model, there was no evidence for increased anxiety levels, or deficits in spatial and learning memory, a measure of the cognitive deficits that characterize schizophrenia, or decreased social interaction, an indicator of depression and/or the negative symptoms of schizophrenia. Pletnikov *et al*.[53] also found increased locomotor activity and unaltered anxiety levels. However, they noted no prepulse inhibition deficits, but did identify deficits in working memory in females only, as well as decreased sociability in males. Thus, these transgenic models display different, but overlapping, phenotypes, each related to aspects of human psychiatric illness, and add further weight to the evidence indicating that altered DISC1 expression predisposes to mental disorders. Moreover, the gender-specific effects identified by Pletnikov *et al*.[53] are consistent with the human genetic studies, indicating that some genetic variants may confer sex-specific risk of developing mental illness.

Although none of these mouse models relate directly to known human causal variants, their phenotypes are in agreement with the human genetic studies that suggest the existence of several mechanisms by which altered DISC1 function can perturb brain function, leading ultimately to mental illness. These, and prospective mice carrying mutations in *Disc1* and in genes encoding its interactors, individually and in combination, promise to provide a powerful platform for investigating the function of DISC1 in relation to major mental illness and for evaluating intervention strategies.

DISC1 gene structure and evolution

The human *DISC1* gene spans approximately 415 kb of genomic DNA (UCSC genome browser, March 2006 assembly) and consists of 13 exons producing a full-length transcript of approximately 7.5 kb.[9,10] Comparative and functional genomics studies have reported conservation of *DISC1* in macaque (*Macaca mulatta*), mouse (*Mus musculus*), rat (*Rattus norvegicus*) zebrafish (*Danio rerio*) and pufferfish (*Fugu rubripes*).[54–57] Additional genome browsing suggests that *DISC1* is also conserved in chicken (*Gallus gallus*), cow (*Bos taurus*) and dog (*Canis familiaris*). There is no obvious insect or worm homologue for *DISC1*.

The genomic structure of *DISC1* is well conserved amongst most species identified to date (Figure 2).

There are however some notable differences: primate *DISC1* appears to differ from rodent and fish, which lack exon 9a of primate *DISC1*;[57] however, more extensive studies are needed to confirm this. Furthermore, pufferfish and zebrafish possess an additional exon 11a that appears to have been lost in mammalian *DISC1* homologues.[57] In mammals, exon 11 is alternatively spliced due to an internal splice donor site. This alternative splicing removes the distal 66 nucleotides of exon 11, but does not disrupt the open reading frame. Exon 11a of pufferfish *DISC1* can also be removed without disrupting the open reading frame; in this sense, the distal part of mammalian exon 11 and exon 11a of pufferfish are analogous.[57] It is therefore plausible that exons 11 and 11a of pufferfish *DISC1* could have merged via intron loss to give rise to the exon 11 we see in mammalian *DISC1* today. Intron 12 of human *DISC1* is of the rare U12-type with AT/AC termini.[10] This intron type is conserved between human, mouse and pufferfish.[10,55,57]

DISC1 intron 9 is surprisingly large (Figure 2). In humans, this intron encompasses approximately one-third of the whole gene at around 140 kb in length. Mouse *Disc1* also possesses a large intron 9 of approximately 50 kb in length (one-quarter of the total gene length) and pufferfish possesses an intron 9 of 4 kb in length (one fifth of the full gene length). Given this conservation of relative size, it is possible that intron 9 possesses some functionality. In humans, this may come in the form of *DISC2*, the antisense RNA gene that overlaps exon 9 of *DISC1*, and with its 5′ located within *DISC1* intron 9, but as yet not formally defined.[9,10]

Human *DISC1* produces at least four known alternatively spliced isoforms; these are denoted Long (L isoform, exons 1–13, AF222980), Long variant (Lv isoform; excludes distal 66 nucleotides of exon 11, AB007926), Short (S isoform; uses an alternative 3′UTR in intron 9 (exon 9a), AJ06177) and Extremely short (Es isoform; contains an alternatively spliced DISC1 exon 1a and terminates transcription two intronic codons after exon 3, AJ506178).[10,57] Rhesus monkey appears to produce L and Lv isoforms, but does not possess S or Es *DISC1* isoforms as judged by reverse transcription-PCR (RT-PCR).[54] EST evidence suggests that at least the L and Lv isoforms are conserved across species.[57] In addition, a novel

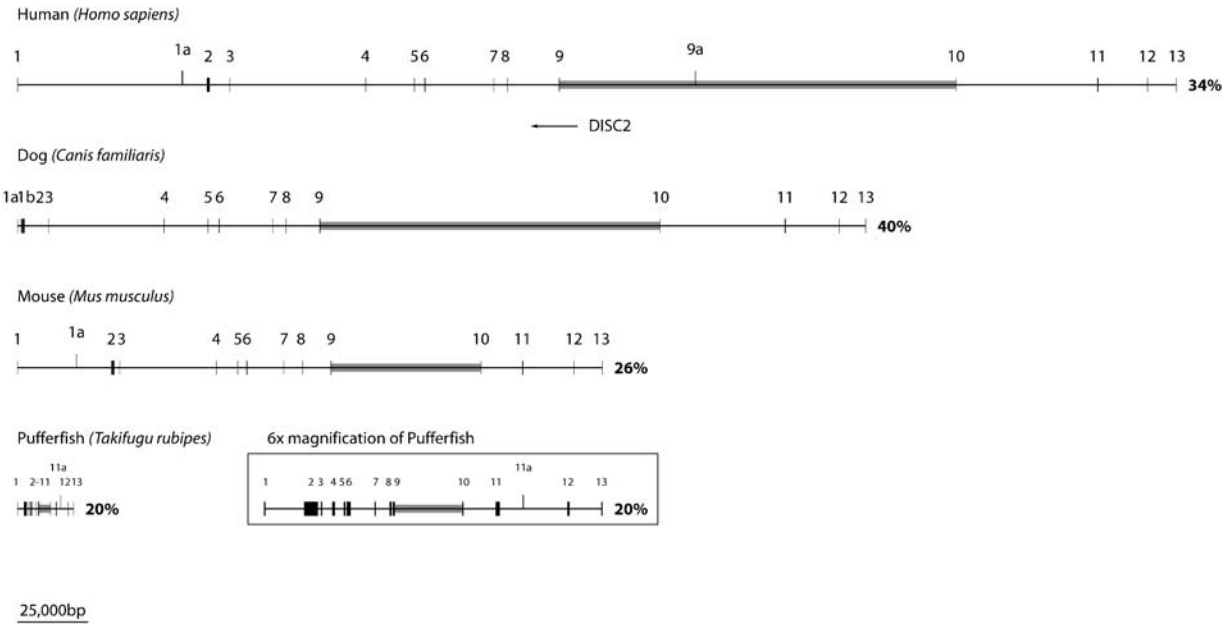

Figure 2 Cross-species conservation of *DISC1* genomic structure. This figure depicts the genomic structure of *DISC1* in human, dog, mouse and pufferfish. The *DISC1* genomic structure for human and mouse was constructed using the previously published data of Millar *et al*.[9] and Ma *et al*.,[55] respectively. The predicted genomic structure of dog *DISC1* was constructed using bl2seq[58] alignments of *Canis familiaris*-predicted mRNA XM546088 with human *DISC1* (AF222980) and the UCSC genome browser BLAT tool.[59] The structure of pufferfish was determined by aligning mRNA AJ496231 to the genomic contig AJ504410 using bl2seq.[58] In humans, the *DISC2* gene is shown overlapping *DISC1* exon 9. To date there is no evidence for this gene is other species. The figure also highlights the exceptionally large *DISC1* intron 9 (grey box) for each species. The percentages represent the proportion of the full-length gene that encompasses intron 9. DISC, Disrupted-in-Schizophrenia.

murine *Disc1* isoform, which results from skipping of exon 9,[55] suggests a further alternative splicing event.

Analysis of *DISC1* mRNA in adult human post-mortem brain samples by quantitative RT-PCR has shown predominant expression of the full-length *DISC1* transcript and low or negligible levels of the alternatively spliced *DISC1* transcripts.[60]

In humans and mice, rare intergenic splicing events between *DISC1* and its neighbouring gene *TSNAX* (translin-associated factor X, Trax) have been demonstrated by RT-PCR.[57,61] The *TSNAX* gene produces a 33 kDa protein that binds and modulates the DNA/RNA binding activity of the nucleic acid binding protein Translin.[62,63] Translin/Trax complexes are highly expressed in brain, where they are implicated in dendritic mRNA translation.[64] These *trans* splicing events are rare and although they produce novel fusion transcripts, they are low in abundance and translation products have yet to be documented.[57,61]

DISC1 protein structure and conservation

DISC1 alternative transcripts give rise to four predicted protein isoforms in humans. The L transcript predicts a full-length protein isoform of 854 amino-acid residues (~100 kDa); the Lv isoform is predicted to be a protein of 832 amino-acid residues (~98 kDa); the S isoform is predicted to contain approximately 678 amino-acid residues (~75 kDa); and the Es isoform is predicted to consist of 369 amino-acid residues (~39 kDa). In agreement with these predictions, DISC1 antibodies in human cells and tissue have detected isoforms of 100, 95–98 and 70–80 kDa.[65] These are in addition to some higher molecular weight isoforms (150 and 200 kDa), which may represent dimers of the various DISC1 protein isoforms.[65] Indeed, DISC1 may exist *in vivo* as a dimer formed via a self-association domain located at amino acids 403–504.[66]

Despite these predictions, correlation of observed isoforms with specific transcripts is incomplete. Three DISC1 isoforms within the 70–80 kDa molecular weight range have been identified.[65] It is likely that one of these isoforms represents the predicted S DISC1 protein isoform. The identity of the other two isoforms is, however, unknown. They may correspond to post-translationally modified or processed forms of the L, Lv or S DISC1 isoforms. For example, James *et al*.[65] reported phosphorylation of an 80 kDa isoform of DISC1, which is in agreement with other studies.[67,68] N-terminal processing of DISC1 may also occur based on the observation that DISC1 isoforms within the 75–80 kDa molecular weight range are commonly detected in studies using C-terminal specific antibodies, which should only detect L or Lv DISC1 isoforms.[69,70] Furthermore, overexpression of C-terminally tagged DISC1 produces tagged DISC1

isoforms of 105, 95, 80 and 74 kDa, suggesting that the full-length protein is N-terminally processed.[71]

The full-length human DISC1 protein (854 aa) is predicted to consist of an N-terminal region, often referred to as the globular 'head' domain (~1–350 aa, encoded by exons 1 and 2) and an alpha-helical coiled–coil-containing C-terminal region (~351–854 aa, encoded by exons 3–13)[9,10] (Figure 3).

From a PROMALS-derived[72] multiple sequence alignment (Figure 4) and calculated pairwise percentage identity values (Table 2), the C-terminal region is noticeably more conserved than the N-terminal region. The N-terminal region does however contain both a conserved putative nuclear localization signal and a short conserved serine/phenylalanine-rich motif that corresponds to a predicted alpha-helix.[54,55,57] Other prominent features within this region include the lack of confidently predicted secondary structure elements, the presence of a few stretches of low-sequence complexity, and compositional bias towards serine, alanine and glycine.[57] The C-terminal region, on the other hand, contains multiple regions with the potential to form coiled–coils (two of which correspond to predicted leucine zipper motifs)[57] (Figure 4). The importance of these coiled–coil regions in facilitating DISC1 protein interactions may explain the evolutionary constraint on the C-terminal region of DISC1; however, although less well conserved, the N-terminal region also participates in a number of protein–protein interactions (Figure 5).

Although the role of *DISC1* was first linked to major mental illness back in 2000,[2] and its importance subsequently confirmed and replicated in numerous independent genetic studies since, no experimental three-dimensional structure has been forthcoming. In fact, to date, any biophysical characterization of the encoded protein is lacking.

DISC1 expression in tissue

Studies at the RNA and protein level have revealed that *DISC1* is expressed in many adult and foetal human tissues including the heart, brain, placenta, kidney and pancreas.[9,65] *DISC1* transcripts are most abundantly expressed in placenta, heart and brain.[9] Heart is also a highly expressing tissue in adult rodents with additional expression documented in brain, kidney, liver and testis.[55,56]

DISC1 expression in adult brain

Northern blotting of human brain tissue has detected widespread *DISC1* expression in multiple brain regions.[9] *DISC1* expression in postmortem human brain is high within the dentate gyrus of the hippocampus,[60,65] accompanied by lower expression in the temporal and parahippocampal cortex.[60] Work has been carried out to define more clearly the regions of *DISC1* brain expression using rodents and monkey as more accessible systems.

In concordance with the human expression data, RNA *in situ* hybridization of adult mouse brain tissue has demonstrated high *Disc1* expression in the dentate gyrus of the hippocampus in addition to low expression in many other regions, including the cerebellum, cerebral cortex, ammon's horn and olfactory bulbs.[55,76] Low expression has also been reported within the paraventricular and arcuate nuclei of the hypothalamus and amygdale.[55] Further studies of rodent *Disc1* by western blotting and immunohistochemistry corroborate these findings.[56,76,77]

RNA *in situ* hybridization of *DISC1* in brains of adult African Green Monkeys also revealed widespread expression including the lateral septum and dentate gyrus of the hippocampus and lower expression within the cerebral cortex, amygdala, cerebellum, paraventricular hypothalamus and the interpeduncular and subthalamic nuclei.[78] A similar expression pattern for *DISC1* has also been reported in Rhesus Monkey by RT-PCR and western blotting.[54]

Thus, the most consistent observation from rodents and primates is prominent expression in the hippocampus. This is an interesting finding, as the hippocampus is strongly implicated in schizophrenia (reviewed elsewhere[79]) and hippocampal lesions in rodents and primates can lead to neuropsychological defects including cognitive, locomotor and behavioural abnormalities similar to those reported in schizophrenia.[80–82] There are, however, regional differences in *DISC1* expression across species. Although this may be due to differences in experimental methods, it is also possible that *DISC1*

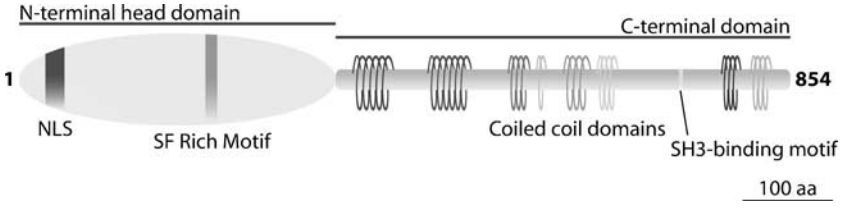

Figure 3 DISC1 predicted protein structure. This figure depicts a cartoon of the predicted structure of the DISC1 protein. This figure was constructed from the data of Millar *et al.*[9,10] and Taylor *et al.*[57] Two conserved regions of the N-terminal region are highlighted; a conserved putative nuclear localization signal (NLS) and a short conserved serine/phenylalanine-rich motif (SF-rich motif). The numerous regions of predicted coiled–coil in the C-terminus are shown, which are likely to be important for mediating DISC1 protein–protein interactions, and an SH3-domain interaction motif is also shown. DISC, Disrupted-in-Schizophrenia.

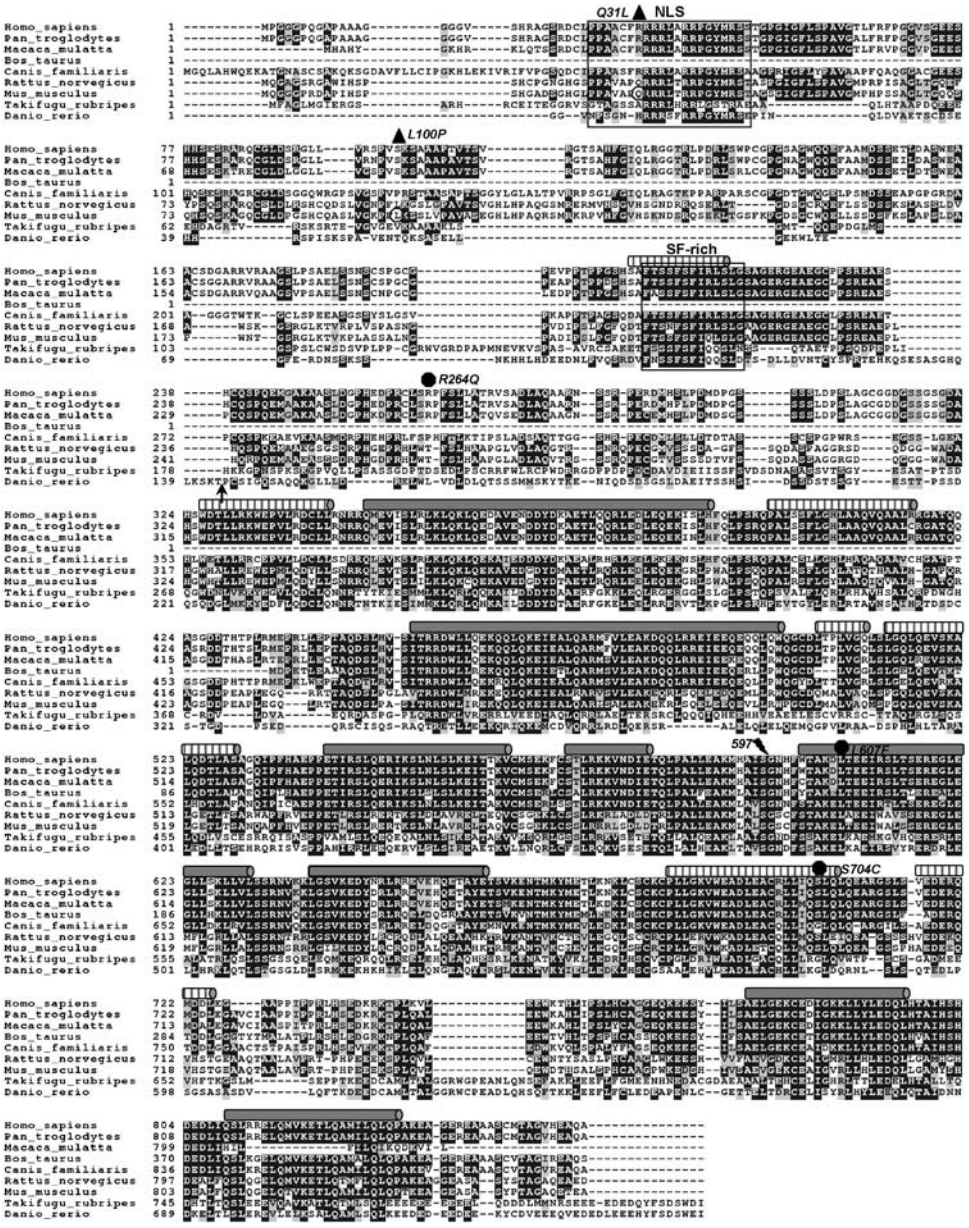

Figure 4 Multiple sequence alignment of DISC1 orthologues. The multiple sequence alignment of DISC1 orthologue sequences was derived using PROMALS.[72] Regions of conservation are shown using BOXSHADE (v3.21) shading (http://www.ch.embnet.org/software/BOX_form.html). The initial alignment was further manually refined based upon conservation of amino acids and optimum matching of predicted secondary structure elements for all sequences. Highly conserved (black background) and conservatively substituted (grey background) residue columns for all sequences are shaded on the alignment. The translocation breakpoint at position 597 (lightning symbol), the putative nuclear localization signal (NLS) and serine-phenylalanine-rich (SF-rich) regions are indicated. Coiled–coil forming potential is shown above the human sequence (bold-shaded tubes), on the basis of Taylor *et al*.[57] and additionally, confident PsiPred-predicted[73] secondary structure α-helices of the human sequence are shown (vertical striped tubes). Known sequence variations for human (R264Q, L607F, S704C; bold shaded octagon) and mouse (Q31L, L100P; bold shaded triangle) are shown. The arrow below *Danio rerio* (block 4 of the alignment) indicates an insertion of 211 amino-acid residues (not shown here) that correspond to several imperfect tandem repeats unique to this protein. These repeats were disregarded for purposes of alignment/analysis and hence the sequence numbering for *Danio rerio* does not reflect its true value. Sequence lengths for the DISC1-orthologues used were as follows: *Homo sapiens* (854), *Pan troglodytes* (858), *Macaca mulatta* (818), *Bos taurus* (420), *Canis familiaris* (886), *Rattus norvegicus* (846), *Mus musculus* (851), *Takifugu rubripes* (807), *Danio rerio* (752). DISC, Disrupted-in-Schizophrenia.

Table 2 DISC1 orthologue pair-wise percentage identity table

	Homo	*Pan*	*Macaca*	*Bos*	*Canis*	*Rattus*	*Mus*	*Takifugu*	*Danio*
Homo	**100**								
Pan	**98** *96, 99*	**100**							
Macaca	**91** *88, 93*	**91** *86, 94*	**100**						
Bos	**81** *0, 81*	**81** *0, 81*	**77** *0, 77*	**100**					
Canis	**71** *58, 81*	**72** *58, 81*	**69** *58, 77*	**81** *0, 81*	**100**				
Rattus	**56** *48, 61*	**56** *49, 61*	**54** *48, 58*	**59** *0, 59*	**51** *43, 57*	**100**			
Mus	**58** *50, 63*	**58** *50, 63*	**56** *48, 61*	**61** *0, 61*	**53** *44, 59*	**84** *81, 86*	**100**		
Takifugu	**34** *27, 38*	**34** *27, 38*	**34** *27, 38*	**37** *0, 37*	**33** *24, 38*	**32** *25, 36*	**32** *25, 36*	**100**	
Danio	**33** *27, 36*	**33** *27, 36*	**33** *27, 36*	**37** *0, 37*	**33** *24, 38*	**30** *23, 33*	**29** *23, 32*	**46** *35, 51*	**100**

The table shows an all-against-all pair-wise percentage sequence identity for DISC1 orthologues. The pair-wise percentage sequence identity (bold number) was calculated using the PIM (percentage identity matrix) option under ClustalX,[74] after providing the final alignment (as in Figure 4) as input. The two values (italics), separated by a comma, show the pair-wise percentage sequence identities for the N-terminus (first value; corresponding to positions 1–350 in human), and C-terminus (second value, corresponding to positions 351–854 in human), respectively, to illustrate the higher conservation of the C-terminus (note *Bos taurus* lacks the N-terminus and some part of its C-terminus).

performs distinct functions in rodent brains to that of the primate brain.

DISC1 expression during brain development

Disc1 is expressed throughout mouse brain development.[83] However, while *Disc1* expression is maintained in the hippocampus throughout brain development in mouse, it is only transiently expressed within the thalamus.[83] *Disc1*-expressing cells in the developing cortex are neuronal in nature and expression is seen in cortical neurons and interneurons at the time of neuronal birth and migration.[77,83] A 100 kDa DISC1 isoform is dynamically expressed during mouse brain development with most prominent expression at embryonic day 13.5 and postnatal day 35, which correspond to periods of active neurogenesis and the onset of puberty, respectively.[77] Ozeki *et al*.[56] have also reported dynamic changes in Disc1 protein expression throughout rat brain development. In human brains, *DISC1* mRNA appears to be more prominently expressed during prenatal, neonatal and pubertal periods than in adulthood.[60] Taken together, these data suggest a role for DISC1 in brain development and maturation.[56,60,77,83] These temporal and spatial changes in DISC1 expression during brain development are likely to affect DISC1 protein interactions.

DISC1 is well placed for a role in schizophrenia considering its widespread expression in the brain (Figure 1d and e), with prominent expression in the hippocampus and other limbic brain regions, and its developmentally regulated expression profile. However, *DISC1* has also been shown to be highly expressed in heart and other tissues from primates and rodents and therefore may play additional important functions in other locations.

Subcellular DISC1 expression in cultured cells

Exogenous and endogenous DISC1 protein expression has been studied in detail in many mammalian cell types, using both primary cells and established cell lines.[56,65–67,69,71,75,76,84–90]

Evidence from studies of endogenous DISC1 strongly supports a cytoplasmic localization and endogenous DISC1 immunoreactivity has been shown to overlap with that of F-actin,[76] α-tubulin,[84,85] MAP2[85] and gelsolin[65] supporting an interaction between DISC1 and the cytoskeleton. Endogenous DISC1 has also been colocalized with markers of the mitochondria[56,65,71,85] and centrosome.[66,75] In primary and established neuronal cell types, DISC1 expression is localized to the cell body, nucleus and neurites[56,65,69,85,87,88] (Figure 1c).

A number of studies have also demonstrated DISC1 expression in growth cones of hippocampal neurons.[76,87,88] Some studies have reported changes in subcellular DISC1 expression, which are related to neuronal differentiation. DISC1 expression is augmented in differentiating rodent (PC12) cells and differentiation coincides with movement of the DISC1 protein from cytoplasmic puncta into neurite-like processes.[56] A similar change in DISC1 distribution

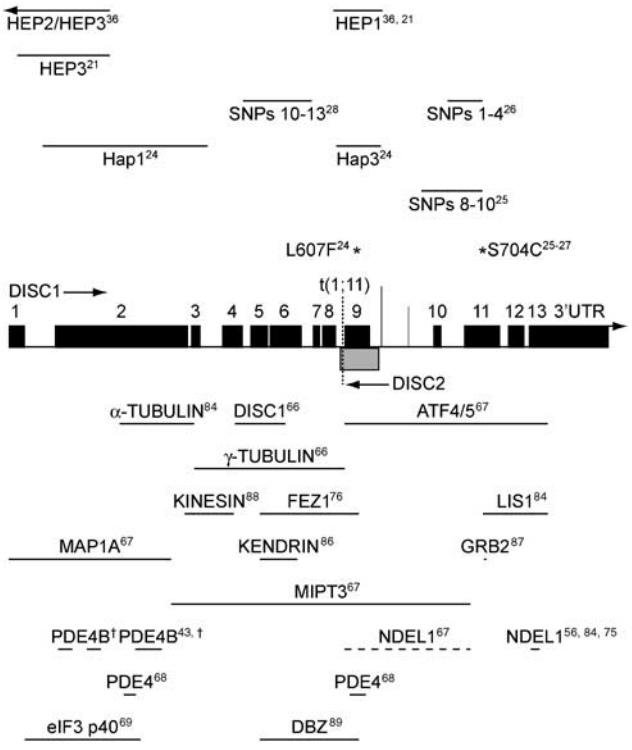

Figure 5 Position of DISC1 protein-binding regions and genetic variants. This figure depicts the regions of DISC1 required for protein–protein interactions and the relationship of the reported genetic variants in DISC1 to these binding regions. The central portion of the figure depicts the exonic structure of *DISC1*. Below the genomic structure is a diagrammatic representation of the DISC1-binding regions required to interact with all of its confirmed protein partners. There are two binding regions reported for the NDEL1 protein, one of which is marked with a dashed line. This dashed region has only been reported in one of four studies; however, it is possible that DISC1 may possess multiple NDEL1-binding regions as has been recently discovered for DISC1 binding to PDE4B (Murdoch *et al.*, in press). Above the genomic structure are the regions of haplotypic and single marker association for the *DISC1* gene in psychiatric illness. Associations were included in the figure based on statistical robustness and/or replication in more than one study. It is notable that the associations and the regions required for protein binding span the full-length of the *DISC1* gene. As such, the regions of protein binding and association show considerable overlap such that genetic variation at the amino acid level may impact upon a multitude of DISC1 protein interactions. The only protein-binding regions not contained within an overlapping haplotype is one of the reported NDEL1-binding regions, the LIS1-binding region and the GRB2 SH3-binding interaction motif; however, it has been reported that the Ser704Cys polymorphism affects DISC1 binding to NDEL1[75] and as it is in even closer proximity to the LIS1- and GRB2-binding regions, it is possible that it may also exert effects on binding of these proteins. References for the data in this figure are denoted by superscripted numbers with the exception of Murdoch *et al.*,[68] which is indicated with an arrow.

has been reported using a human neuroblastoma cell line (SH-SY5Y).[65]

It is clear that DISC1 is localized to many subcellular compartments, suggesting that it may have multiple roles in various locations throughout the cell (Table 3a, Figure 1c). Different DISC1 protein isoforms may therefore perform distinct protein interactions as a result of preferential localization to a particular subcellular compartment. There already exists some evidence that DISC1 protein isoforms exhibit differential subcellular localization. For example, the 71 kDa human DISC1 protein isoform has been shown to be present exclusively within the mitochondrial fraction, while the longer isoforms assume nuclear, cytoplasmic, cytoskeletal and centrosomal locations.[65]

DISC1 subcellular expression by fluorescence microscopy has mainly been described as punctate, granular or aggregate within the cytoplasm, and filamentous in association with the mitochondria.[65,67,71,76,84–86] In contrast, C-terminally truncated forms of DISC1 are commonly reported as diffusely localized within the cytoplasm.[56,66,67,71,85] Amino acids 1–358 (the 'head' domain) of DISC1 are important for DISC1 localization to the nucleus,[71] and regions within the C-terminal tail of the protein are particularly important for DISC1s cytoskeletal and centrosomal localization, as DISC1 truncates lacking portions of the C-terminus generally exhibit diminished localization to the cytoskeleton and centrosome[66,67,71,85,86] (Table 3b).

A recent study of DISC1 immunoreactivity at the ultrastructural level in the human brain has shown that in the human neocortex DISC1 is expressed within cell bodies and apical dendrites of pyramidal neurons, glial cells and also in scattered neuronal-like cells of the white matter.[91] By electron microscopy, DISC1 expression was found to be associated with axon terminals, the post synaptic density and dendritic spines. Intracellular immunoreactivity was shown to be commonly associated with ribosomes within the dendritic shafts and the cell body. Additional DISC1 immunoreactivity was noted with regions of chromatin and the endoplasmic reticulum.[91] Intriguingly, this study did not document DISC1 localization at the mitochondria, one of the most prominent sites of expression;[65] however, this is likely due to the experimental procedures and the particular DISC1 antibody used.

Overexpression studies have given insights into potential functions of DISC1 within the cell. Millar *et al.*[71] reported that overexpressing DISC1, particularly C-terminally truncated forms, results in a mitochondrial ring phenotype, suggesting that it may influence mitochondrial fission and fusion. Additionally, Ogawa *et al.*[69] have shown that overexpression of DISC1 induces the assembly of stress granules containing both DISC1 and eukaryotic translocation initiator factor 3 (eFI3), suggesting that DISC1 may have a role in translation in response to environmental stress.

Table 3 Subcellular localization of the DISC1 protein

(a) Subcellular localization of full-length DISC1

Cell lines	*Cell body (cytoplasmic or cytoskeletal)*	*Mitochondrial*	*Nuclear*	*Perinuclear*	*Centrosomal*	*Cellular processes (includes neurites + growth cones)*
Endogenous DISC1						
PC12	56, 87, 88			56, 75	75	56, 87, 88
SH-SY5Y	84, 65, 69,85	65, 85	65, 69			69
U373-MG	65	65	65			
COS7	71	71	71	66	66	
SK-N-SH	76			76		
Rodent neurons	85, 88, 87	85		84	66	76, 85, 88, 87
HeLa	56	56		56		
Exogenous DISC1						
SH-SY5Y	86, 69, 67			67	67, 86	
Rodent neurons	85					85, 76
HeLa	86, 85					
COS/COS7	71	71	71	66, 56	71, 86	
PC12	80			90	90, 66	90
NT2N neurons	67			67		67

(b) Subcellular localization of exogenous DISC1 truncates

Cell lines	*DISC1 construct*	*Cytoplasmic diffuse*	*Cytoplasmic punctate*	*Mitochondrial*	*Nuclear*	*Centrosomal*	*Cellular processes*
NT2N neurons	1–597	67	67				67
SH-SY5Y	1–597	67	67			67	67
SH-SY5Y	FL (missing 446–533)	86					
COS/COS7	1–597	71, 66	56	71	71		
COS7	1–358	71		71	71		
COS	291–854	56					
HeLa	FL (missing 446–533)	86					
HeLa	598–854	85					
HeLa	541–854	85					
HeLa	440–854		85				
HeLa	357–854		85				
HeLa	440–540	85					
HeLa	440–597		85				
PC12	1–597	90					
PC12	788–849	75					
Rodent neurons	1–597	88					88
Rodent neurons	1–361	88					88

Abbreviation: DISC, disrupted-in-schizophrenia.

This table documents the subcellular expression of DISC1 as detected by immunofluorescent microscopy in primary or established cell lines. The cell lines studied are listed in the left-most column, and the subcellular regions reported are listed along the top row. The numbers indicate the references, which have reported each finding. Part (a) reports the location of the full-length DISC1 protein either by examining endogenous or exogenous expression. Part (b) documents the subcellular localization of truncated forms of the DISC1 protein. The full-length DISC1 protein is seen to occupy many locations within the cell and is associated with the cytoskeleton, the mitochondria and the centrosome. Some truncated forms of DISC1 fail to assume the correct subcellular distribution, often being reported as diffusely localized within the cytoplasm and failing to associate with subcellular structures. However, in some cases truncated DISC1 isoforms do assume a normal distribution, including the mitochondria and centrosome, but their level of expression is diminished in these regions.

DISC1 expression in psychiatric illness

To date, few studies have examined the expression of *DISC1* in patients with psychiatric illness, presumably due to the lack of patient material. One study using quantitative RT-PCR analysis of *DISC1* in postmortem brain samples (Clinical Brain Disorders Branch, NIMH) reported no difference in expression of full-length *DISC1* transcripts between schizophrenia and controls samples, within the hippocampus or dorsolateral PFC.[60] However, analysis of DISC1 by immunoblotting in these brain samples detected one major band of 70–75 kDa, which exhibited a trend towards increased expression (approximately 20%) in the hippocampus of schizophrenia patients compared to controls.[60] This is in contrast to unchanged *DISC1* mRNA levels, and intriguingly this protein isoform does not correspond to the predicted size of the full-length *DISC1* transcript predominantly expressed in these brains. These data suggest potential post-translational regulation of DISC1 expression and again points towards likely post-translational processing or modification of full-length DISC1 in concordance with previously published data.[65,67,71]

Using postmortem brains received from the Stanley Foundation,[92] Sawamura *et al*.[70] have shown that total brain homogenates do not show altered DISC1 expression between schizophrenia, bipolar, major depressed and control subjects. However, crude subcellular fractionation of these homogenates revealed an imbalance of DISC1 expression in the nuclear and postnuclear fractions in schizophrenia and major depression compared to controls.[70]

Although it is desirable to study *DISC1* expression directly in the brains of affected individuals, human brain tissue is not easily accessible, and therefore, surrogates in the form of cells are necessary to analyse potential pathological changes in *DISC1* expression and function. Lymphoblastoid cell lines are easily attainable from patients and provide an infinite source material in which gene expression can be analysed. Two studies have utilized such lymphoblastoid cell lines to examine DISC1 expression.

In cell lines derived from members of the t(1;11) family, in which the *DISC1* gene was first identified,[9] *DISC1* gene expression has been shown to be decreased at both the mRNA and protein level in translocation carriers compared to normal karyotype controls.[43] This evidence favours a haploinsufficiency model of causation, and is supported by RNA interference experiments for DISC1, as outlined in subsequent sections.[27,66,75,87,88]

DISC1 mRNA was also shown to be decreased in lymphoblastoid cells derived from bipolar subjects in comparison to control unaffected family members.[93] This decreased expression was associated with a 12 marker *DISC1* risk haplotype spanning the full-length of the *DISC1* gene;[93] however, this broad haplotype is most likely a surrogate for an as yet unidentified risk variant. Decreased *DISC1* expression was also correlated with the number of manic symptoms in these patients.[93]

If alterations in *DISC1* expression, as suggested by these studies, contribute to the pathogenesis of schizophrenia, one might also expect antipsychotic medication to influence *DISC1* expression. In human postmortem samples, antipsychotic medication was not seen to affect *DISC1* expression.[60] However, administration of antipsychotic medication at clinically relevant doses to mice does result in upregulation of *DISC1* expression within the frontal cortex and hippocampus.[94]

The current data regarding *DISC1* expression in psychiatric illness are limited and preliminary at best. There is no clear pattern of altered *DISC1* expression, and postmortem studies to date have given mixed results, which may be due to differences in patient samples and preparation of source brain material. Furthermore, although lymphoblastoid cell lines have shown reductions in *DISC1* expression in both bipolar disorder and the Scottish translocation family, there are limits to the use of these transformed cell lines in analysing gene expression. However, altered *DISC1* expression is not a pre-requisite to implicate this gene in psychiatric illness, as in some cases, the function of the protein may be altered independently of changes in gene expression.

DISC1 protein interactions

Studies of protein complexes involving DISC1 have proved particularly fruitful in furthering our understanding of its function. A very large number of potential DISC1 interactors have been identified[43,56,67,76,84,86–88,95,96] (Figure 5) and the majority can be loosely classified into the following groups: cytoskeleton, cell cycle, signal transduction, intracellular transport/exocytosis, golgi and central nervous system (CNS) development. These classifications clearly demonstrate that DISC1 is likely to be important in several diverse and critical brain functions. However, very few of the potential interactions have been investigated in any detail so far, and most were selected for further study due to their known neuronal function or involvement in the aetiology of psychiatric illness. This review will focus only upon those interactors that have been studied beyond their initial identification as putative DISC1 binding partners.

PDE4B as a risk factor for psychiatric illness: interaction with DISC1

PDE4B is a member of the PDE4 family, consisting of four genes (PDE4A-D) that altogether specify at least twenty distinct protein isoforms. PDE4s possess cAMP hydrolysis activity that is specifically inhibited by the prototypical antidepressant drug rolipram (for a review, see Houslay and Adams[97]). They have a modular structure consisting principally of an N-terminal domain that is believed to direct subcellular targeting, two regulatory regions referred to as upstream conserved regions (UCR1 and UCR2) and a

catalytic domain (for a review, see Houslay and Adams[97]). A combination of multiple promoters and alternative splicing dictates inclusion of specific N-terminal domains, and the presence or absence of UCR1 and UCR2 sequences, to create PDE4 isoforms that are likely to be targeted to particular cellular compartments and discretely regulated in response to cAMP levels (for a review, see Houslay and Adams[97]). This diversity of PDE4 isoforms is believed to underpin compartmentalized cAMP signalling, which is critical for CNS functioning.

Mammalian PDE4s are orthologous to the *Dunce* gene of *Drosophila melanogaster*, and studies of *Dunce* mutants have provided insights into the likely function of PDE4s in the brain. Mutations in *Dunce* cause learning and memory defects, which result from altered cAMP hydrolysis and associated elevated cAMP concentrations.[98] It has also been shown that *Dunce* mutants have altered axonal growth cone motility,[99] neuronal function[100,101] and synaptic plasticity.[102] The PDE4 inhibitors MEM1018 and MEM1091 influence NMDA receptor-dependent memory,[103] and other selective PDE4 inhibitors including rolipram have been shown to influence memory and cognition in several studies.[103–110] Moreover, translation of specific PDE4 isoforms is regulated during long-term potentiation[111] and in NMDA-receptor-mediated memory functions.[112] PDE4s also influence myelination,[113] and rolipram has been shown to reduce demyelination and CNS inflammation,[114] and to promote axon regrowth and myelination *in vivo*.[115]

The PDE4B gene is directly disrupted by a chromosomal translocation in a patient diagnosed with schizophrenia and a cousin with psychosis. This translocation also disrupts the cadherin 8 gene (CDH8), implicating both genes as risk factors for major mental illness.[43] Subsequent genetic studies focusing on the PDE4s have demonstrated association between PDE4B and schizophrenia in the populations of Scotland[116] and Finland.[117] Interestingly, genetic association between schizophrenia and PDE4D has also been detected in Finnish samples,[117] suggesting a wider involvement of PDE4s in the pathology of psychiatric illness. This identification of PDE4s as genetic risk factors for schizophrenia is intriguing, as it suggests that aberrant cAMP signalling and the CNS processes identified as defective in *Drosophila Dunce* mutants may also be deficient in some psychiatric patients.

Consistent with the emerging evidence for an involvement of PDE4s in mental illness, mice deficient in PDE4B or PDE4D behave as if they have been treated with antidepressants.[118,119] Indeed, several antidepressants are believed to act on independent pathways that ultimately converge on the cAMP system,[120,121] and synaptic plasticity is modulated by many drugs used to treat mood disorders.[122,123] Moreover, the PDE4 inhibitor rolipram produces behavioural effects similar to those resulting from antipsychotic treatment of rats and mice,[124,125] and this antipsychotic action is mediated by PDE4 isoforms.[125] Thus, there is potential for PDE4s to be involved in both mood and psychotic disorders.

The PDE4s are abundantly expressed in the CNS and exhibit isoform-specific expression,[126–130] suggesting that each isoform has a specific role within the brain. Sites of expression include many regions implicated in the pathology of mental illness, such as the hippocampus, frontal cortex and nucleus accumbens. Several studies have reported that treatments for psychiatric disorders modulate PDE4 expression in some of these areas of the brain: antidepressant treatments including electroconvulsive shock and various medications alter expression in many brain regions.[131–134] In addition to the studies of PDE4s in brain, PDE4 expression is modulated by lithium in lymphoblastoid cell lines derived from lithium-responsive patients with bipolar disorder.[135] These effects may be interpreted as meaning that the PDE4s participate in responses to therapeutic treatments, or alternatively, as providing further confirmation of PDE4 involvement in causing illness. Nicotine has also been shown to influence brain expression of PDE4s, causing dose-dependent decreases in the hippocampus, PFC and nucleus accumbens.[136] This is an intriguing observation given the very high rate of nicotine addiction amongst psychiatric patients, and prevailing theories of nicotine use as a form of self-medication.

The genetic, functional and biochemical data supporting involvement of the PDE4s in mental illness are thus persuasive, but the demonstration of direct binding of the PDE4s to DISC1[43] provides additional compelling evidence. The regulatory UCR2 domain of PDE4 isoforms interacts with the N-terminal head domain of DISC1[43] and additional contact sites between the two proteins have since been identified (Murdoch *et al.*, in press). Since the UCR2 domain is common to each of the four PDE4 genes, DISC1 is able to bind to PDE4A, B, C and D isoforms, although demonstration of interaction between endogenous proteins has so far been limited to the 71 kDa DISC1 isoform and PDE4B.[43] Intriguingly, binding between these endogenous proteins is dynamic and dependent upon cellular cAMP levels, with PDE4B dissociating from 71 kDa DISC1 in response to elevated cAMP. Under these same conditions, PDE4B catalytic activity increases. These observations support a model where DISC1 sequesters PDE4B in a low-activity form until cAMP signalling is switched on, whereupon PDE4B is released in a catalytically active form to hydrolyse cAMP and switch off the signalling cascade.[43] However, this simple model is complicated by our recent observations that the dynamic association is DISC1 and PDE4 isoform-specific (Murdoch *et al.*, in press) and it is clear that there is potential for complex compartmentalized regulation of cAMP signalling through this interaction. Further potential intricacy is added by observations that reduction of DISC1 expression using RNA interference results in reduced levels of active extracellular signal-related kinase (ERK) in cultured

rat cortical neurons[27] and at axonal growth cones of neurotrophin-treated cultured rat hippocampal neurons,[87] which may in turn influence PDE4 activity, because ERK phosphorylates, and regulates the activity of, the PDE4 catalytic domain.[137] It is clear that the function of DISC1/PDE4 complexes is likely to be elaborately regulated at many levels and, since both DISC1 and PDE4B have been independently identified as risk factors for major mental illness, it is possible that disturbance of any aspect of this interaction may be an important factor in the aetiology of disorders such as schizophrenia. It remains to be seen how much of PDE4 function involves DISC1, but since DISC1 contains five separate contact sites for PDE4B, of which four encompass a large proportion of the N-terminal head domain (Murdoch *et al.*, in press), it is likely that DISC1 function is closely tied up with that of PDE4B.

DISC1/PDE4 complexes may consequently provide new opportunities for therapeutic intervention. Inhibition of PDE4 activity by the antidepressant drug rolipram suggests that there is potential for treatment of psychiatric disorders through direct modulation of PDE4 activity. Rolipram is not in clinical use, because its ingestion is associated with unacceptable side effects. However, diazepam is a drug with clinical efficacy that inhibits the activity of PDE4s when overexpressed in cultured HEK293 cells.[138] Several other specific PDE4 inhibitors are either available or in development and these may ultimately provide the basis for novel therapeutic strategies designed to treat the symptoms of major mental illness. Direct modulation of the interaction between DISC1 and PDE4 isoforms is another possibility for future clinical intervention. Thus, if their involvement in the aetiology of psychiatric illness is confirmed, DISC1 interaction with the PDE4 family of isoforms may present an exciting opportunity to improve the outlook for many psychiatric patients.

LIS1, NDEL1, NDE1 and DISC1

Lissencephaly is a severe brain malformation resulting from defective neuronal migration. In Miller–Dieker syndrome, lissencephaly results, in part, from disruption of the *LIS1* gene.[139,140] In the absence of the Miller—Dieker syndrome, chromosomal deletion, mutations in LIS1 cause a less severe disorder termed isolated lissencephaly sequence (for a review, see Kato and Dobyns[141]).

Mice with mutations in one copy of the *Lis1* gene display defects in cortical layering, cell packing, neuronal migration and neuroblast proliferation.[142,143] Adult *Lis+/−* mice display ectopic neurogenesis within the dentate gyrus. These ectopic cells exhibit abnormal proliferation, a lower survival rate and impaired differentiation, suggesting that haploinsufficiency for *LIS1* may result in abnormal neurogenesis within the adult hippocampus.[144]

LIS1 is one component of a conserved nuclear distribution (nud) pathway that was identified in *Aspergillus nidulans*. The *Aspergillus* orthologue of LIS1, NUDF,[145] functions within a complex of NUD proteins, including NUDA (dynein heavy chain), NUDG (dynein light chain), NUDK (dynactin subunit Arp1) and NUDE.[145–149] The composition of this complex is conserved in mammals, demonstrating that nuclear positioning is an indispensable function. *Aspergillus* NUDE has two mammalian orthologues termed NUDE and NUDE-like (NDE1 and NDEL1), which interact with LIS1.[149–151] Although NDE1 and NDEL1 are highly homologous and expressed in both proliferating neuroblasts and migrating neurons,[151,152] they are likely to be functionally distinct, since NDE1 is expressed most abundantly during early embryonic stages, while NDEL1 expression peaks later in development.[150,152] As with LIS1, mutations in NDE1 and NDEL1 cause defective neurogenesis and neuronal migration.[153,154]

The role of mammalian othologues of the NUD proteins in neuronal migration may be due to their function in nuclear positioning. In mammals, nuclear positioning is critical in the process of neuronal migration, which occurs as a stepwise process. First, migrating neurons extend a leading process (neurite) guided by chemoattractants and chemorepellants. This is followed by translocation of the centrosome and then the nucleus into the leading process (nucleokinesis), and subsequent retraction of the trailing process. The centrosome may play a critical role in determining the direction of migration, and in nuclear translocation into the leading process (for reviews, see Badano *et al.*,[155] Lambert de Rouvroit and Goffinet,[156] Tsai and Gleeson[157]). LIS1, NDEL1 and NDE1 localize to the centrosome[150–153] and in cells deficient for LIS1, dynein or NDEL1 the distance between the nucleus and centrosome is abnormally large.[158–160] This decoupling of the centrosome from the nucleus appears to be due to loss of a specific microtubule bundle known as the microtubule fork/cage, which connects the two organelles together,[158,159] and is essential for nuclear migration.[161] Disruption of this structure and therefore nucleus–centrosome coupling may be one means by which LIS1/NDEL1 knockdown inhibits nuclear migration.

LIS1 and NDEL1 also influence microtubule stability and organization[154,158,160,162,163] and are involved in microtubule-based transport in both anterograde and retrograde directions.[151,164] Knockdown of NDEL1 expression using RNA interference blocks neurite production,[75] which may result from defective tubulin cytoskeletal dynamics and/or deficient microtubule-based transport to the growing neurite tip.

The effect of altering expression of these proteins on neuronal migration may therefore be due to their combined roles in neurite outgrowth, maintaining nuclear–centrosome attachment, as well as the critical importance of creating, maintaining and adapting the microtubule cytoskeleton for all steps of migration.

Defective neurogenesis in *Lis1*, *Ndel1* and *Nde1* mutant mice may arise from their role in regulating the cell-cycle and mitosis. Faulkner *et al.*[165] observed

that LIS1 expression levels correlate with the length of the cell cycle. In mammals, *Drosophila* and *Caenorhabditis elegans* LIS1, NDEL1 and NDE1 are all required for formation of the mitotic spindle,[165,166] while LIS1 and NDEL1 are also implicated in checkpoint control of the cell cycle.[167] It is therefore possible that these proteins influence cortical development by modulating neuronal proliferation in the ventricular zone,[156] as well as by regulating migration of newborn neurons from the ventricular zone to the cortical plate.

Association of DISC1 with LIS1, NDEL1 and related proteins was discovered when a number of yeast two-hybrid screens identified NDE1, LIS1, dynein and dynactin as potential binding partners.[56,67,84] Subsequent studies have confirmed the association between DISC1 and these proteins, which can all be co-immunoprecipitated together from rat PC12 cells,[56,66,67,84] with the exception of NDE1, which has not yet been investigated. However, the DISC1 binding region of NDEL1 is conserved in NDE1 and it is assumed that NDE1 is a binding partner also.

NDEL1 interacts with specific DISC1 isoforms, since the Lv DISC1 variant, which lacks 22 amino acids encoded by exon 11,[57] exhibits impaired NDEL1 binding,[75] implying that this particular isoform may possess alternative functions within the cell. Moreover, additional DISC1 isoforms such as the S and Es variants lack C-terminal sequences,[57] including the region responsible for binding LIS1, and a leucine zipper which interacts with NDEL1.[56,67,75,84]

Intriguingly, in mouse brain binding between NDEL1 and DISC1 is developmentally regulated, with a strong association detectable from embryonic day 17 to postnatal day 7, which subsequently decreases from postnatal day 16 to adulthood at which point it is virtually undetectable.[84] This interval overlaps substantially with the period of active neuronal migration within the developing cortex.

DISC1 localizes to the centrosome,[66,67] the key organelle for control of microtubule dynamics, and also associates with microtubules,[67,84,85] suggesting it may have a key role in LIS1/NDEL1-related microtubule dynamics. Furthermore, overexpression of a C-terminally truncated form of DISC1 termed mutDISC1 (which lacks the NDEL1- and LIS1-binding region[56,67,75,84]), disrupts the accumulation of LIS1, NDEL1, dynein and dynactin at the centrosome.[66] As with NDEL1, DISC1 also associates with γ-tubulin[66] and binds directly to pericentrin B,[86] and may thus be critical for anchoring LIS/NDEL1 protein complexes and microtubules to the centrosome. Consistent with an involvement of DISC1 in microtubule anchoring and dynamics, expression of mutDISC1 results in gross disorganization of the microtubule network and delayed microtubule aster formation following treatment of cells with nocodazole, a microtubule depolymerizing agent.[66]

Through its interaction with NDEL1 and LIS1, DISC1 is implicated in mitosis, neurite outgrowth and neuronal migration. Consistent with this, the large-scale yeast two-hybrid study recently reported by Camargo *et al*.[95] identified mitosis as an aspect of cellular function in which DISC1 is likely to play a significant role. DISC1 is also likely to be critical for neurite production, since overexpression of C-terminally truncated Disc1 or knockdown of Disc1 in rat PC12 cells inhibits neurite outgrowth,[56,66] while DISC1 overexpression has the opposite effect.[76] In addition, Disc1, like Ndel1, is upregulated during nerve growth factor-stimulated PC12 cell differentiation, when neurite extension occurs extensively.[75] Moreover, fragments of DISC1 containing the NDEL1-binding site inhibit Disc1 interaction with Ndel1 and block neurite outgrowth, suggesting that DISC1/NDEL1 interaction is required for neurite production.[75] DISC1 is also critical for neuronal migration since knockdown of mouse Disc1 *in-utero* at embryonic day 14.5 inhibits the migration of neurons from the ventricular zone to the cortex that should be detectable by postnatal day 2.[66] By postnatal day 14, the few neurons that have migrated are incorrectly positioned and exhibit abnormal dendritic arborization.[66] There is also evidence that *in utero* Disc1 knockdown correlates with decreased nucleus–centrosome coupling,[66] similar to that seen for LIS1 and NDEL1,[158] suggesting that DISC1 is another critical component of the conserved nuclear migration pathway.

In mouse brain *Disc1*, *Ndel1* and *Lis1* expression overlaps within the developing cerebral cortex[77,83,152,168,169] and other regions, including the hippocampal dentate gyrus.[55,77,83,168,170] These proteins are all present at the post-synaptic density,[91,150] while Lis1 and DISC1 are also present at additional synaptic locations,[91,171] suggesting roles in synaptic transmission.

LIS1 and NDEL1 possess additional functions, since LIS1 binds to catalytic subunits of platelet-activating factor acetylhydrolase[172] and NDEL1 has oligopeptidase (EOPA) activity,[173] which is responsible for the metabolism of certain neuropeptides.[174,175] While DISC1 has no known role in the activity of platelet-activating factor acetylhydrolase, EOPA activity is inhibited by DISC1 overexpression,[173] thus implicating DISC1 in the regulation of NDEL1 oligopeptidase activity, and hence in neuropeptide metabolism.

Although pathways involving LIS1, NDEL1, NDE1 and DISC1 are implicated in psychiatric illness, genetic evidence for involvement of *LIS1* is lacking. However, genetic variants located within the lissencephaly critical region that encompasses *LIS1* have been discovered in schizophrenia and bipolar disorder patients,[176] while studies of mRNA levels in postmortem brain from schizophrenia patients do implicate *LIS1*, as well as *NDEL1*.[60] Moreover, there is also evidence that *NDEL1* is associated with schizophrenia in the Finnish population.[117] In addition, a recent study reported linkage to *NDE1* in schizophrenia samples of Finnish origin, which had been stratified on the basis of containing the schizophre-

nia-associated HEP3 risk haplotype in *DISC1*.[177] This study also found evidence of association between *NDE1* and schizophrenia in females.[177]

DISC1 interaction with 14-3-3ε and kinesin-1

Kaibuchi and Colleagues[87] have recently used an affinity chromatography approach coupled with mass spectrometry, to identify DISC1-binding partners from rat brain cytosol or membrane fractions. Several DISC1-associated proteins from the cytosol extract were identified by mass spectrometry, including a kinesin-1 heavy chain (KIF5B) and 14-3-3ε.[88]

14-3-3 proteins represent approximately 1% of total soluble brain protein. They are critical for many brain functions, and are involved in several neurological diseases. 14-3-3 proteins are present in the neurofibrillary tangles associated with Alzheimer's disease[178] and may facilitate Tau hyperphosphorylation which contributes to tangle formation.[179,180] 14-3-3 proteins, including 14-3-3ε, also interact with α-synuclein[181] and may contribute to the pathogenesis of Parkinson's disease. Spinocerebellar ataxia type 1 results from the accumulation of mutant ataxin-1 containing polyglutamine tracts[182] and 14-3-3 isoforms, including 14-3-3ε, are believed to take part in this process.[183] 14-3-3 proteins may also be involved in additional neurological disorders, such as Creutzfeldt-Jakob disease (for a review, see Dougherty and Morrison[184]).

In addition, 14-3-3ε is deleted in Miller-Dieker syndrome, a disorder that includes severe neuronal migration defects, resulting in part from mislocalization of NDEL1 and LIS1 due to loss of 14-3-3ε binding to NDEL1.[185] 14-3-3ε thus complexes with LIS1 and NDEL1 *in vivo* and is responsible for maintaining their correct localization. Following their demonstration that DISC1 associates with 14-3-3ε, Taya *et al*.[88] demonstrated that many proteins were present in the DISC1 affinity column eluate in addition to KIF5B and 14-3-3ε, including LIS1 and NDEL1. DISC1 associates with kinesin and LIS1/NDEL1/14-3-3ε in rat PC12 cells, and in rat hippocampal neurons these proteins partially colocalize at axonal growth cones in association with microtubules.[88] RNA interference experiments demonstrated that accumulation of 14-3-3ε, LIS1 and NDEL1 at the growth cones is dependent upon the presence of DISC1 and kinesin. This study additionally demonstrated that DISC1 and kinesin are essential for axon outgrowth, and therefore suggests that DISC1 functions in trafficking essential proteins to the axonal growth cone to facilitate axon outgrowth. Taya *et al*.[88] thus propose that DISC1 acts as the cargo receptor for the 14-3-3ε/LIS1/NDEL1 complex *in vivo*.

The work of Taya *et al*.[88] suggests that 14-3-3ε may be involved in molecular mechanisms that underlie mental illness, and 14-3-3 proteins, although not 14-3-3ε to date, have indeed been implicated by some studies. There is evidence for genetic association of the 14-3-3η and ζ genes with schizophrenia.[186–189] Moreover, expression of several 14-3-3 genes is decreased in PFC of patients with schizophrenia.[190] In addition, expression of some 14-3-3 genes is sensitive to drugs used to treat psychiatric illness, since 14-3-3β is upregulated in PFC of monkeys treated with the neuroleptic haloperidol[190] and levels of 14-3-3δ and 14-3-3ζ are altered in rat cortical neurons in response to application of the antidepressant fluoxetine.[191]

DISC1 interaction with Grb2

Kaibuchi and Colleagues[87] used the same affinity chromatography approach to identify growth factor receptor-bound protein 2 (Grb2) as a robust DISC1 interactor from rat brain membrane extracts. Interestingly, the binding site for Grb2 on DISC1 is very close to that for NDEL1 and Shinoda *et al*.[87] demonstrated that Grb2 competes with NDEL1 for binding to DISC1. DISC1 therefore associates either with NDEL1 or Grb2 *in vivo* and these two interactions are thus functionally distinct.

In axonal growth cones of rat hippocampal neurons, DISC1 and Grb2 are associated with microtubules and DISC1 knockdown experiments using RNA interference showed that this localization of Grb2 is DISC1 dependent.[87] Moreover, DISC1 and Grb2 complex with kinesin (KIF5A) *in vivo*, and kinesin knockdown experiments demonstrated that kinesin is essential for Grb2 motility and accumulation within axonal growth cones.[87] Thus, DISC1 is required for kinesin-dependent movement of Grb2 along microtubules. This study also demonstrated that DISC1/Grb2 interaction is required for neurotrophin (NT-3) induced axonal elongation.[87]

However, Grb2 is involved in several additional cellular functions throughout the body,[192] linking cell surface receptors to several intracellular signalling pathways. A full overview of Grb2 function is beyond the scope of this review; however, a number of interesting observations serve to illustrate the important involvement of this protein in many aspects of CNS function. Grb2 binds several brain proteins, including the dopamine receptors DRD3 and DRD4,[193,194] huntingtin[195] and PDE4D4.[196] Grb2 may also be involved in the pathology of Alzheimer's Disease through binding the amyloid precursor protein (APP)[197] and APP-processed fragments.[198,199] This latter interaction is detectable in human brain and is upregulated in postmortem brain tissue from Alzheimer's Disease cases.[200] Interestingly, Grb2 also binds presenilin 1 (PS1), which is involved in cleavage of APP.[201] Grb2, APP and PS1 reportedly associate at the centrosome, where the complex targets extracellular signal-related kinases 1 and 2 signalling.[201] Finally, it is particularly intriguing that robust interactions between Grb2 and ErbB receptors have been demonstrated. ErbB receptors bind neuregulin and are thus already suspected to be involved in molecular pathways that underlie some cases of severe psychiatric illness (for a review, see Corfas *et al*.[202]). In the CNS, neuregulin stimulates recruit-

ment of ErbB4 and Grb2 to neuronal lipid rafts.[203] Grb2 involvement in neuronal signalling is thus directly modulated by neuregulin, and DISC1 function may turn out to be similarly influenced by neuregulin and vice versa through its interaction with Grb2.

DISC1 interaction with FEZ1

Fasciculation and Elongation Factor Zeta 1 (FEZ1) is an orthologue of the *C. elegans* protein UNC-76 that is critical for neuronal function, since it is involved in axon outgrowth and bundling. Loss of UNC-76 function in nematodes results in defective axonal transport and locomotion that can be rescued by FEZ1.[204] In rat brain, FEZ1 is expressed primarily in neurons in a pattern closely paralleling that of DISC1,[205] and in rat hippocampal neurons reduced FEZ1 expression induced by RNA interference results in several neuronal defects affecting neuronal polarity and axon growth, as well as intracellular transport.[206] The latter observation is consistent with demonstrations of an involvement of FEZ1 in kinesin-based motility.[207] When the proteins are overexpressed, DISC1 binds robustly to FEZ1, and these proteins colocalize in growth cones of cultured rat hippocampal neurons.[76] The interaction is upregulated during differentiation of rat PC12 cells, and differentiated PC12 cells overexpressing DISC1 exhibit enhanced neurite production, which involves DISC1/FEZ1 complexes.[76] Interaction between DISC1 and FEZ1 awaits confirmation with endogenous proteins *in vivo*, and we therefore do not yet know how much of FEZ1 function involves DISC1, but the available data clearly implicate DISC1 in critical neuronal functions which, if adversely affected, are likely to have important consequences for brain function.

Independent genetic evidence for FEZ1 as a schizophrenia susceptibility gene is weak, with only one of three studies[208–210] reporting nominal significance for two polymorphic markers in the Japanese population.[210] The additional two studies reported negative associations for FEZ1 in Japanese, North American Caucasian and African American populations.[208,209] Expression of *FEZ1* mRNA is however significantly reduced in postmortem brain samples from subjects with schizophrenia.[60]

DISC1 interaction with ATF4 and ATF5

The interaction between DISC1 and activating transcription factor 4 (ATF4) has been studied in very little detail so far, and is limited to identification of ATF4 as a potential DISC1 interactor in yeast two-hybrid screens and confirmation of the interaction using a mammalian two-hybrid system.[67] However, a recent demonstration that expression of a truncated form of DISC1 in rat PC12 cells causes redistribution of nuclear ATF4 is encouraging as it suggests that there is indeed an interaction between these two proteins.[90] Nevertheless, ATF4 is worthy of mention, because it is a cAMP-responsive transcription factor whose expression and transcriptional activity is regulated in human cancer cells by the heregulin splice form of neuregulin (NRG1).[211] Thus, it is conceivable that like Grb2, ATF4 may link DISC1 and NRG1 functions in the brain.

ATF4 has many other diverse cellular roles including regulation of presenilin 1 expression, developmentally regulated metabotropic GABA(B) receptor binding[212] in mouse brain and cultured neurons,[213–215] and regulation of GABA(B) promoter activity.[216] DISC1 may therefore influence neuronal signalling via its interaction with ATF4. Intriguingly, the Aplysia homologue of ATF4 has been referred to as a 'memory suppressor gene'[217] and mice with reduced ATF4 expression have enhanced hippocampal synaptic plasticity and memory storage.[218] Thus, interaction with ATF4 provides one possible mechanism by which DISC1 may modulate hippocampal function, aspects of which have been reported to be influenced by DISC1 genotype.[25]

ATF5 is another cAMP-responsive transcription factor that interacts with DISC1.[67] The function of ATF5 within the CNS is not well studied, but it has been shown to influence differentiation of neural progenitors[219,220] and proliferation and differentiation of oligodendrocytes.[221] Moreover, ATF5 is one of the 13 genes found to be differentially expressed in lymphoblastoid cells from two pairs of monozygotic twins discordant for bipolar disorder,[222] suggesting a possible involvement for ATF5 in the aetiology of mood disorders.

Both ATF4 and ATF5 are also involved in modulating responses to cellular stress, and ATF4 expression is upregulated in response to activation of numerous stress response pathways (for a review, see Rutkowsky and Kaufman,[223] Al Sarraj *et al*.,[224] Watatani *et al*.[225]).

There is a lack of genetic data for ATF4 and ATF5 in relation to psychiatric illness; however, one study has reported a nominal association for ATF4 with bipolar II patients of Japanese ethnicity, although this association did not withstand correction for multiple testing.[226] Furthermore, this study did not find differential expression of ATF4 or ATF5 in lymphoblastoid cell lines derived from BPI patients from their Japanese cohort.[226]

Other DISC1 interactors identified in yeast two-hybrid screens

DISC1 interactions with microtubule-associated protein 1A (MAP1A) and TNF receptor-associated factor 3 interacting protein 1 (TRAF3IP1/MIP-T3) have also been explored and confirmed using exogenously overexpressed proteins.[67] Of these proteins, MAP1A is of particular interest, because it is expressed primarily in neurons where it is required for axon and dendrite development (for a review, see Halpain and Dehmelt[227]). MAP1A is most abundantly expressed in adult brain in humans[228,229] and is important for regulating microtubule stability. How-

ever, despite its name, MAP1A also associates with, and crosslinks actin fibres.[230] As well as DISC1, MAP1A-binding partners include the post-synaptic density protein PSD-95.[231]

Interaction with a zinc–finger protein designated DISC1-Binding Zinc–finger protein (DBZ) was recently identified.[89] DBZ, also known by other names including Su48 or talanin, is expressed highly in brain, in a pattern overlapping that of DISC1.[89] Interaction between endogenous DISC1 and DBZ is robust and reduced by the neurotrophins NGF and PACAP (pituitary adenylate cyclase activating polypeptide 1). DISC1 binding to DBZ influences neurite extension in PC12 cells.[89] Intriguingly, Su48 complexes with NDE1 at the centrosome[232] and a number of studies provide suggestive evidence that this gene may be involved in major mental illness.[233–237]

Finally, a novel function for DISC1 is suggested by its interaction with EIF3S3, a component of the eukaryotic initiation factor 3 complex. Association of DISC1 with this complex has been confirmed *in vivo*, implying a novel role for DISC1 in translational regulation of protein production.[69]

DISC1 pathway interactors and major mental illness

Analysis of the very large number of putative DISC1 interactors identified to date (Figure 5) suggests that DISC1 acts as a hub, linking many different functional complexes within the brain. Emerging themes include cAMP signalling, centrosomal and microtubule-based functions, kinesin-mediated intracellular transport and neurite extension. The potential crosstalk with pathways involving other candidate genes such as neuregulin, and dysbindin, which share multiple putative binding partners with DISC1,[95] is intriguing. This may all explain, in part, why mutations within DISC1 are sufficient to cause substantially elevated risk of developing major mental illness in humans, and related phenotypes in mice, despite the extensive genetic evidence pointing towards a polygenic basis for psychiatric illness. It is likely that DISC1 mutations have this effect, because DISC1 is a hub protein and multiple systems critical to CNS development and function are affected as a consequence. Thus, DISC1 may be regarded as a gene of major effect, although estimates of its prevalence in psychiatric illness vary. Other essential hub proteins may similarly turn out to be genes of major effect, and another well-replicated risk factor for severe psychiatric illness, NRG1, is involved in many diverse aspects of CNS function, including neuronal migration, myelination and NMDA receptor signalling (for a review, see Britsch[238]). It follows therefore, that in some cases where DISC1 is not directly implicated, variants acting on its binding partners and other risk factors may act instead to confer risk of schizophrenia and depression, and as we have discussed there is indeed evidence to implicate many DISC1-binding partners as risk factors for mental illness.

Disrupted-in-Schizophrenia-2 (DISC2)

The *DISC2* gene is also disrupted by the t(1;11) translocation and should therefore also be considered as a candidate gene for major mental illness. *DISC2* is located on the opposite strand to *DISC1* and is transcribed in a distal to proximal orientation thus representing a natural antisense transcript (NAT) partially overlapping *DISC1*.[9] *DISC2* overlaps *DISC1* exon 9 with the, as yet undefined 5′ end, thought to lie within the very large intron 9 of *DISC1*.[9,57] The proportionately large size of this intron is conserved across species, suggesting that intron 9 sequences may be functionally important. In humans, this may be because this intron harbours the *DISC2* gene; however, no species conservation has been detected for *DISC2* to date.[9,57] It is possible therefore that *DISC2* is a primate (or human) specific gene. Expression of *DISC2* has been reported predominantly in human heart, where it produces multiple splice isoforms; however, it is expressed in brain at low levels.[9]

Although genetic evidence for the *DISC* locus is strong, with many positive linkage and association peaks across the length of the gene (Table 1), these studies do not discriminate between *DISC1* and *DISC2* in terms of positive linkage and association; furthermore, many studies report markers that are within the *DISC2* gene region.[13,14,24,36] Therefore, *DISC2* may represent an important schizophrenia candidate gene in its own right.

The central dogma states that DNA gives rise to RNA which gives rise to protein. However, we now know that this is not necessarily the case and that not all RNA gives rise to protein, nor does RNA need to code for protein to possess functionality. It has become apparent in recent years that noncoding RNA molecules are abundant within the human genome and noncoding RNAs make up the vast majority of the eukaryotic transcriptome.[239]

A subgroup of putative regulatory RNA molecules that are becoming increasingly interesting are the NATs. *DISC2* appears to belong to this class of noncoding RNA molecules, as it is located antisense to *DISC1* and lacks any significant coding potential.[9] Many eukaryotic genes are known to have associated NATs; these include genes involved in regulation of development, cell-cycle, apoptosis and translation.[240] NATs are believed to function as negative regulators of their protein-coding counterparts. There are a variety of ways in which NATs could regulate sense mRNA expression. They may regulate the expression of sense mRNAs at the level of transcription via competition for regulatory factors, or through physically hindering the progress of transcription, either topologically or by being transcribed themselves.[241] They may also regulate sense gene expression post-transcriptionally by binding and blocking regulatory sites in the sense mRNA. This can potentially regulate any step in RNA processing including translation, polyadenylation, splicing, transport or even degrada-

tion.[241] Moreover, sense:antisense dsRNAs can also result in RNA editing or activation of cellular siRNA-related pathways resulting degradation of homologous transcripts and gene silencing.[241,242]

To date, the importance of non-coding RNA genes has been underestimated and they have been overlooked in favour of protein-coding genes; however, antisense transcription within the human genome has been implicated in the pathogenesis of many diseases. Of particular interest in relation to psychiatric illness is their implication in neurological disorders (for a review, see Costa[243]).

Considering the importance of 'noncoding' RNA molecules, it is an exciting possibility that *DISC2* functions as a noncoding RNA molecule. The fact that it is antisense to *DISC1* opens up the possibility that it may act as a riboregulator of *DISC1*, and therefore it is plausible that disruption of *DISC2* might have a role in the pathological mechanism of schizophrenia through dysregulation of *DISC1* expression. It may also be the case, however, that *DISC2* functions independently of *DISC1* and that its function may be independently important in the pathogenesis of schizophrenia.

Summary

Studies of DISC1-interacting proteins have greatly added to our knowledge and understanding of DISC1 biology over the last few years. Many important interactions have been defined and genetic variations in DISC1 that may affect these interactions (Figure 5) have been shown to impact upon cognitive function and normal cognitive ageing. Although DISC1s implication in psychiatric illness is now strongly supported by both genetics and biology, the mechanism by which DISC1 contributes to the pathogenesis of psychiatric illness remains unknown. There is no clear pattern of altered *DISC1* expression or function in psychiatric illness and much more work is needed to explore this. However, this is just one of the many questions that remain to be answered in relation to the *DISC* locus and psychiatric illness.

Remaining questions

As with any new field, no sooner is it established than the unanswered questions soon exceed the starting evidence. This is certainly true for *DISC1*. We do not yet know the genetic architecture and distribution of risk and how (or whether) this correlates with prognosis or response to drug treatment. The evidence for the striking phenotypic differences between the two missense *Disc1* mutant mouse lines suggests that the same might be so in human subjects in relation to normal cognitive variation, psychopathology, prognosis and response to treatment. With various *Disc1* mouse models waiting in the wings, there will be an inevitable pressure to report novel features, but it will be every bit as important to establish commonalities and distinguishing features for core behavioural, pharmacological and biochemical assessments.

DISC1 exhibits widespread expression within the CNS and also in peripheral tissues; however, remarkably little is known about the regulation of DISC1, the determinants of isoform expression or of post-translational modification. All are likely to be crucial in determining DISC1 function. Little research has been invested into elucidating the structure and function of *DISC1*'s antisense partner, *DISC2*, which may be of paramount importance in regulating gene expression at this locus, at least in primates, and therefore, by inference, may be important in fine-tuning the higher level developmental and cognitive aspects of DISC1 function.

We know that DISC1 occupies multiple subcellular compartments and there is evidence for both cooperative and competitive binding with its partners. We know that DISC1 produces many protein isoforms, however, the cloning and molecular characterization of only two of these has been carried out to date. Understanding the isoform specificity as well as the temporal and spatial control of these protein interactors is challenging, but important. That DISC1 has no similarity to other known proteins means that we have only a rudimentary and incomplete prediction of domain structure. Solving the structure of DISC1 and predicting the effect of mutations is a high priority. Some clues about the function and role of DISC1 have come from the intrinsically limited studies of human DISC1 in the brain. Perhaps the emerging mouse models can help here in refining specific questions that can then be tested in the human. Only a small fraction of the potential DISC1 interactors have been formally confirmed, but the large-scale yeast two-hybrid study of Carmargo *et al*.[95] suggests that we should be looking at an effect of DISC1 on mitosis, the cytoskeleton and intracellular transport. It will be valuable to determine whether supportive evidence for this emerges from genome-wide expression studies in appropriate model systems. Again, the mouse models are well suited to such hypothesis generation, as the developmental stage, cell type and the influence of various interventions, such as environmental deprivation or enrichment, or drug treatment are all controllable. Human brain studies of genome-wide expression are fraught with difficulties because of the uncertainties of tissue provenance. However, a recent study of gene expression profiles in autism[244] gives encouragement to the notion that comparison of gene expression profiles in lymphoblastoid cell lines between cases and controls, stratified on DISC1 variants, may be productive.

Despite a clear link between the biology of the DISC1 protein, nervous system development and function, DISC1 is not exclusively brain expressed. To our knowledge, however, the nature of illness caused by haploinsufficiency of DISC1 overtly affects only higher mental functions. Although perplexing, DISC1 is not alone in this respect, as a number of genes implicated in organic diseases of the brain are

also expressed ubiquitously, yet specifically affect brain function. One example is the product of the *IT15* gene (*interesting transcript 15*), the huntingtin protein (Htt).[245] Htt is ubiquitously expressed yet the mutant form of the htt protein, which results from a polyglutamine (CAG) repeat expansion,[245] specifically causes a neurodegenerative disorder of the brain, which affects projections neurons of the striatum and cortex resulting in movement disorders (chorea), cognitive dysfunction and manifestations of a psychiatric nature (for a review, see Li and Li[246]).

Is the discovery of DISC1 and the DISC1 pathway a real breakthrough in mental health research? Can genetic variation in DISC1, and hence function, explain the subtle neuropathology of schizophrenia, core cognitive dysfunction and psychosis experienced by sufferers, either alone or in combination with additional known or novel risk factors? To address this matter, it is necessary to examine all components of the DISC1 pathway in detail. Is DISC1 the most susceptible link, genetically or biologically? Would the phenotype of genetic variation in for example PDE4B plus NDEL1 and/or LIS1 be equivalent or distinct in phenotype from downregulation of DISC1? Does DISC1 itself or other pathway proteins provide tractable targets for drug development or non-pharmacological modulation of therapeutic value?

Perhaps it is too early to answer any of these questions, but the combined genetic and biological approach, which has characterized the DISC1 field to date is essential if progress is to be made. In that regard, the DISC1 field has established a paradigm of integrated genetic and biological analysis of candidate genes. The field of molecular psychiatry can and must move beyond LOD scores, *P*-values and odds ratios to mutations, mechanisms and interventions.

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Note added in proof

The generation and integration of new neurons in the adult hippocampus is an important form of structural plasticity in the mammalian brain. Strikingly, in a recent paper in cell, Duan *et al*.[247] have identified Disc1 as an important mediator of neuronal integration in the adult hippocampus. The authors used stereotaxic surgery to inject retroviral vectors coexpressing GFP and Disci shRNA into proliferating neural progenitor cells in the adult mouse hippocampus. While there was no effect on neuronal fate specification of adult neuronal progenitors, it was demonstrated that Disc1 knockdown results in overextended migration of newborn neurons, soma hypertrophy, increased dendritic initiation and arborization, increased synapse formation and abnormal electrophysiological properties, suggestive of accelerated maturation of these neurons. Complimentary experiments on Ndel1 knockdown suggest that Disc1 and Ndel1 interact synergistically to control the processes of migration and integration of adult-born neurons in the mouse hippocampus.

These data demonstrate that Disc1 may have complex alternative functions during embryonic and adult brain development and maturation, as it has been previously reported by Kamiya *et al*.[66] that knock-down of Disc1 during embryonic brain development in mouse inhibits neuronal migration and dendritic arborization in the developing cortex, while the opposite occurs during adult neurogenesis.

The hippocampus and hippocampal neurogenesis are strongly implicated in schizophrenia and depression, and in the therapeutic action of many drugs used to treat depression. This study by Duan *et al.*,[247] therefore gives us new insight into the mechanisms by which Disc1 might increase susceptibility to psychiatric illness and may also help to explain the adult onset of schizophrenia and related conditions.

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