Dissecting DISC1 function through protein–protein interactions

N.J. Brandon

Schizophrenia and Bipolar Research, Wyeth Discovery Neuroscience, CN8000, Princeton, NJ 08543, U.S.A.

Abstract
Disrupted in schizophrenia 1 (DISC1) is emerging in the eyes of many as the most promising candidate of all the schizophrenia risk genes. This viewpoint is derived from the combination of genetic, clinical, imaging and rapidly advancing cell biology data around this gene. All of these areas have been reviewed extensively recently and this review will point you towards some of these excellent papers. My own personal view of the potential importance of DISC1 was echoed in a recent review which suggested that DISC1 may be a ‘Rosetta Stone’ for schizophrenia research [Ross, Margolis, Reading, Pletnikov and Coyle (2006) Neuron 52, 139–153]. Our own efforts to try to understand the function of DISC1 were through identification of its protein-binding partners. Through an extensive Y2H (yeast two-hybrid) and bioinformatics effort we generated the ‘DISC1-Interactome’, a comprehensive network of protein–protein interactions around DISC1. In two excellent industry–academia collaborations we focused on two main interacting partners: Ndel1 (nudE nuclear distribution gene E homologue-like 1), an enigmatic protein which may have diverse functions as both a cysteine protease and a key centrosomal structural protein; and PDE4B, a cAMP-specific phosphodiesterase. I will work the research around these two protein complexes in detail.

Introduction
It is clear that schizophrenia has high heritability, and in the last decade, a large number of putative risk genes have been identified. Of these, DISC1 (disrupted in schizophrenia 1) now has one of the most compelling stories through a combination of clinical, genetic and functional studies [1–3]. Until very recently, the biological function of DISC1 was very poorly understood. We decided to investigate the function of DISC1 though identifying its protein interacting partners and discovering their functional significance. Two distinct methodologies were used: Y2H (yeast two-hybrid) screening and a mouse brain co-immunoprecipitation/MS analysis approach. The overlap in candidate partners between these two approaches was not high. The MS data have yet to be published and will not be discussed further. An initial set of Y2H screens with DISC1 resulted in the identification of a group of proteins of high interest. A number of bioinformatic- and literature-driven decisions were then made to follow up a select number of these proteins with additional Y2H screens. Analysis of these screens resulted in the construction of the DISC1 interactome: a highly connected network consisting of 127 proteins and 158 interactions [4]. Understanding the cellular role of DISC1 has been accelerated though protein interaction studies including linking DISC1 to cAMP signalling, axon elongation and neuronal migration. All of these breakthroughs have been derived from identifying protein interaction partners of DISC1 [Ndel1 (nudE nuclear distribution gene E homologue-like 1), PDE4B (phosphodiesterase 4B), kinesin motor proteins] and analysing the function of the resultant complexes [5–7]. All of these interactions, and many more besides, can be found in the DISC1 interactome [4].

The DISC1 and Ndel1/Nde1 (nuclear distribution gene E homologue 1) partnership
The best characterized of the DISC1 protein–protein interactions so far is that with Ndel1/Nudel (Nudel, nuclear distribution element-like; EOPA, endo-oligopeptidase A), the latter term is not widely used but was suggested by our group several years ago [19]. Ndel1 and its orthologue Nde1 had been identified through their interaction with the molecule Lis1, mutations in which are known to underlie human lissencephaly [8–12]. These mutations are thought to disrupt neuronal migration causing malformations in the layering of the cortex, resulting in a ‘smoothened brain’. Causative mutations in Lis1 were shown to inhibit binding to Ndel1. Ndel1 has since been shown to play a role in a range of cellular functions. For example, Ndel1 has been shown to modulate dynein function and is vital for the coupling of the centrosome and nucleus during neuronal migration and in determining neuronal positioning [13]. Meanwhile Nde1/Nudel has been shown to play a key role in centrosome duplication and mitotic spindle assembly, which in an Nde1-KO (knockout) mouse manifested itself as microcephaly with reduced progenitor cell division and altered neuronal cell fate [10]. It is clear that these proteins are critical for

Key words: disrupted in schizophrenia 1 (DISC1), nudE nuclear distribution gene E homologue-like 1 (Ndel1), oligopeptidase, phosphodiesterase (PDE), schizophrenia.

Abbreviations used: DISC1, disrupted in schizophrenia 1; ENU, N-ethyl-N-nitrosourea; EOPA, endo-oligopeptidase A; KO, knockout; Nde1, nuclear distribution gene E homologue 1; Ndel1, nudE nuclear distribution gene E homologue-like 1; PDE4B, phosphodiesterase 4B; PKA, protein kinase A; RNAi, RNA interference; Y2H, yeast two-hybrid.

1email brandon@wyeth.com
Furthermore, there are three possible cysteine residues involved in Ndel1, Cys203 and Lys266/Glu267. Predictions that this molecule would also have some very interesting properties. We were able to show that in the developing post-natal mouse brain, the interaction can only be detected by conventional co-immunoprecipitation studies for a distinct time period; namely E17 (embryonic day 17) through to P16–P21 (postnatal days 16–21). Beyond this age the ability to detect the interaction decreases and, by conventional techniques, was not detectable at 6 months of age [16]. Intriguingly, we have some preliminary correlative evidence that the mechanism underlying this developmental control is phosphorylation of Ndel1 (N.J. Brandon, unpublished work). Other groups have recently focused their efforts on the characterization of the DISC1–Ndel1 complex at the centrosome, in particular by a very elegant set of experiments by Sawa and co-workers [6]. They utilized in vitro RNAi (RNA interference) to deplete DISC1, resulting in abnormal neuronal migration, thinning of dendritic spines and a decreased number of dendritic arborizations [6].

Our own interests in this complex were diverted by the realization that Ndel1 was the same protein as EOPA, a putative cysteine protease, which had been shown to degrade a wide range of important neurotransmitters in vitro [18]. In collaboration with the group of Antonio Camargo and Mirian Hayashi, we investigated the oligopeptidase activity of Ndel1 and the importance of this activity in whole rat brain. Cloning of human EOPA showed that it was identical with human Ndel1. We were able to confirm by pharmacological manipulations that human Ndel1 was a cysteine protease. Furthermore, there are three possible cysteine residues in Ndel1, Cys273, Cys275 and Cys347, which could form the active site. We individually mutated these three residues to alanine and showed that mutation of Cys273 abolished enzymatic activity completely [19]. Utilizing a blocking antibody which specifically inhibits Ndel1 activity, we were able to show that, in rat brain, Ndel1 contributes to 50% of overall oligopeptidase activity. Using gel-filtration chromatography, we could show that the enzymatically active form of Ndel1 in this preparation was the monomer [19]. This observation is clearly very interesting as it suggests that binding of Ndel1 to other proteins may inhibit its enzymatic activity.

This hypothesis gained support as we completed our understanding of the interaction domains with the identification of the site on Ndel1 where DISC1 binds. Initial Y2H and comprehensive pull-down data with truncated forms of Ndel1 and point mutations showed that Leu266 and Glu267 on Ndel1 were both required for the interaction to occur. The spatial proximity of the two sites (Cys273 and Lys266/Glu267) suggested that the interaction may modulate enzymatic activity. Indeed, incubation in vitro of Ndel1 with DISC1 competitively inhibits the oligopeptidase activity [19]. The full biological relevance of this aspect of the DISC1–Ndel1 partnership is still unknown and there are currently many unanswered questions. For example, to date, all of the experiments have been performed in vitro and actual enzymatic activity in cells has not yet been shown. Also the role of the peptidase activity in neuronal migration is not yet clear. Although simple sequence analysis of the orthologue Ndel1 shows that it retains the binding site at Lys266/Glu267 and the enzymatically important Cys273, predictions that this molecule would also show enzymatic activity have not been shown to date.

**DISC1 and PDE4**

One of the most exciting and novel partners that we identified from the Y2H screen was PDE4B, a member of a family of enzymes which regulate levels of cAMP [4], work that was completed as part of a collaboration with the groups of David Porteous and Miles Houslay. There was immediate interest in this finding, as pharmacological and KO mouse data had previously implicated PDE4 in various psychiatric disorders [20,21] and PDE4 had been the subject of drug-discovery efforts for a range of other therapeutic indications [22]. There are four PDE4 genes, which form more than 20 different splice variants through the use of alternative start sites and alternative splicing [23]. Each gene has so-called long, short and super-short forms, depending on whether they contain the regulatory regions termed UCR1 and 2 in the N-terminus. Long forms have a UCR1 and UCR2 domain, where UCR1 can be phosphorylated at a single critical serine site by PKA (protein kinase A) within the consensus RRESF, leading to a conformational change in UCR2, enhancing its PDE activity [23]. Short forms do not contain UCR1, while super-short lack UCR1 and have a truncated UCR2 domain. We characterized the interaction between DISC1 and PDE4 using PDE4B1 (long), PDE4B3 (short) and PDE4B2 (long). All of these isoforms were able to bind DISC1 and, by further refined mapping experiments, we were able to show that the UCR2 domain was critical for binding [5]. Interestingly, the UCR2 domain has also been shown to bind the centrosomal protein myomegalin [24], and also DISC1 has been identified as a centrosomal protein and so it will be interesting to explore the dynamics of these complexes. As indicated above, PKA phosphorylation of UCR1 leads to increased enzyme activity, thus allowing the cell to maintain a homeostatic cAMP environment. Previously PKA phosphorylation was shown to cause a disruption in the interaction between UCR1 and UCR2, and so the role of phosphorylation in modulating the DISC1–PDE4 complex was analysed. In SHSY5Y neuroblastoma cells, PKA phosphorylation of UCR1 was shown to cause dissociation of the DISC1–PDE4B complex. The model, as originally proposed, suggests that DISC1 binds to a non-PKA-phosphorylated form of PDE4, whereupon cAMP increases and subsequent...
activation of PKA leads to the release of an activated pool of PDE4B (Figure 1).

The Porteous and Houslay groups have made great progress around this story in the last 2 years, after the collaboration with Merck ended, and recently there has been publication of two DISC1 mutant mouse lines generated by ENU (N-ethyl-N-nitrosourea) mutagenesis in conjunction with the Roder group [25]. The mice have single point mutations in the N-terminus of DISC1. I will not go into the behavioural phenotypes here, but, in summary, the mutant line Q31L is claimed to have a ‘depressive-like’ phenotype and L100P to have a more ‘schizophrenic’ phenotype. The potential connection with PDE4 and DISC1 comes from an earlier finding that PDE4 binds to the N-terminus of DISC1, and in particular that residues 219–283 were important, but recent unpublished work, alluded to in the mouse paper, states that there are also regions in DISC1 overlapping the mutations Q31L and L100P that are important for the interaction. In HEK-293 (human embryonic kidney) cells, both mutations decrease binding of DISC1 to PDE4B1 and B3. The data for a decrease are far more convincing for the L100P mutation. The molecular prediction would be that in the L100P animals you might expect to see an increase in PDE activity due to the decreased interaction, but instead a 50% decrease in activity was observed in the Q31L mice, with no change in L100P. The pharmacology backs up this enzymology, as Q31L animals are resistant to the effects of rolipram (a PDE4 inhibitor that raises cAMP levels) to reverse some of the strain’s behavioural deficits.

The data from the ENU mice clearly reinforces the point that the DISC1–PDE4 complex could be of great importance for the aetiology of psychiatric diseases. During the original study, the balance was tipped to follow this interaction over others, as, in parallel, Millar et al. [5] had identified a schizophrenia patient with a balanced translocation in the PDE4B gene. A cousin of the patient also had the same translocation and a psychotic illness. This discovery made it compelling to follow up the DISC1–PDE4B interaction in detail.

**DISC1–PDE4–NDEL1: an unlikely triumvirate**

Although we do not have data to show how the DISC1–PDE4 and DISC1–Ndel1 complexes interrelate, we have proposed a potential model for this (Figure 1). We have already mentioned that, in SHSY5Y cells under basal conditions, DISC1 binds PDE4 in a low-activity state, and in particular that residues 219–283 were important, but recent unpublished work, alluded to in the mouse paper, states that there are also regions in DISC1 overlapping the mutations Q31L and L100P that are important for the interaction. In HEK-293 (human embryonic kidney) cells, both mutations decrease binding of DISC1 to PDE4B1 and B3. The data for a decrease are far more convincing for the L100P mutation. The molecular prediction would be that in the L100P animals you might expect to see an increase in PDE activity due to the decreased interaction, but instead a 50% decrease in activity was observed in the Q31L mice, with no change in L100P. The pharmacology backs up this enzymology, as Q31L animals are resistant to the effects of rolipram (a PDE4 inhibitor that raises cAMP levels) to reverse some of the strain’s behavioural deficits.

The data from the ENU mice clearly reinforces the point that the DISC1–PDE4 complex could be of great importance for the aetiology of psychiatric diseases. During the original study, the balance was tipped to follow this interaction over others, as, in parallel, Millar et al. [5] had identified a schizophrenia patient with a balanced translocation in the PDE4B gene. A cousin of the patient also had the same translocation and a psychotic illness. This discovery made it compelling to follow up the DISC1–PDE4B interaction in detail.

**Figure 1 | Hypothetical model of DISC1–PDE4–Ndel1 complexes**

A hypothetical cellular model of the interaction of the complexes between DISC1 and either PDE4 or Ndel1. On the left-hand side, under conditions of low cAMP, DISC1 binds and holds PDE4 in a state of low activity. Ndel1 is not DISC1-bound, so is active. Increases in cAMP, on the right-hand side, lead to dissociation of DISC1–PDE4 and possibly creation of DISC1–Ndel1 complexes with a concomitant inhibition in Ndel1 activity, paralleled by increases in PDE activity. L273, key catalytic centre; UCR1/2, regulatory domains of PDE4; c1–5, coiled-coil domains of DISC1.
Summary
Protein–protein interactions have allowed for dramatic progress in the understanding of the function of DISC1, and although Y2H screens are often maligned for their potential to throw up false positives, in this case, the technique has been well used. The daunting thing is that only a handful of the hundreds of DISC1 interactome proteins have been followed up. It will be critical to use more efficient technologies to follow up the individual interactors and also the whole system as we try to see whether DISC1 can live up to its billing as the ‘Rosetta Stone for schizophrenia’ [1].

References

Received 11 June 2007
doi:10.1042/BST0351283