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Review

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Revisiting Disrupted-in-Schizophrenia 1 as a scaffold protein

Abstract: *Disrupted-in-Schizophrenia 1* (*DISC1*) is a widely-accepted genetic risk factor for schizophrenia and many other major mental illnesses. Traditionally DISC1 has been referred to as a 'scaffold protein' because of its ability to bind to a wide array of other proteins, including those of importance for neurodevelopment. Here, we review the characteristic properties shared between established scaffold proteins and DISC1. We find DISC1 to have many, but not all, of the characteristics of a scaffold protein, as it affects a considerable number of different, but related, signaling pathways, in most cases through inhibition of key enzymes. Using threading algorithms, the C-terminal portion of DISC1 could be mapped to extended helical structures, yet it may not closely resemble any of the known tertiary folds. While not completely fitting the classification of a classical scaffold protein, DISC1 does appear to be a tightly regulated and multi-faceted inhibitor of a wide range of enzymes from interrelated signaling cascades (Diverse Inhibitor of Signaling Cascades), which together contribute to neurodevelopment and synaptic homeostasis. Consequently, disruption of this complex regulation would be expected to lead to the range of major mental illnesses in which the *DISC1* gene has been implicated.

Keywords: DISC1; mental illness; protein-protein interactions; signaling pathways; structure; threading models.

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DISC1 – a major gene involved in behavioral control and chronic mental illness

Disrupted-in-Schizophrenia 1 (*DISC1*) is one of the highest profile risk factors for schizophrenia and other chronic mental illnesses (Chubb et al., 2008) having originally been identified as a gene that was disrupted by a chromosomal translocation (1;11) strongly linked to psychiatric illness in a large Scottish family (Millar et al., 2000; Blackwood et al., 2001). Since then, a substantial body of genetic evidence has accumulated, supporting the notion that *DISC1* is more generally associated with multiple chronic mental illnesses including schizophrenia, depression and autism (reviewed in: Chubb et al., 2008; Bradshaw and Porteous, 2012), as well as with cognitive dysfunction in healthy individuals (Callicott et al., 2005; Thomson et al., 2005). The concept of *DISC1* being a key player in behavioral control in supported independently by several rodent models expressing mutant or deleted variants of the *DISC1* gene (Shen et al., 2008; Brandon and Sawa, 2011; Kuroda et al., 2011).

The concept of DISC1 as a scaffold protein

Initial analysis of the amino acid sequence of the DISC1 protein yielded no clear indications as to its function beyond a similarity to certain structural proteins (Millar et al., 2000) and to date no evidence of enzymatic activity has been reported. However, yeast two-hybrid studies implicated the DISC1 protein in binding to numerous protein interaction partners (Millar et al., 2003; Miyoshi et al., 2003; Morris et al., 2003; Ozeki et al., 2003) using multiple distinct binding sites (reviewed in: Soares et al., 2011). Together this led to the suggestion that DISC1 is 'a multifunctional protein, which interacts via distinct domains with different components of the intracellular

machinery' (Morris et al., 2003) and which 'is likely to be critical to development and functioning of the brain by acting as a scaffold to bring together certain proteins, such as signal transduction molecules' (Millar et al., 2003). This was reinforced by further systematic yeast two-hybrid screening, contributing greatly to a list that now numbers more than 200 potential DISC1-interacting proteins, the 'DISC1 interactome', and the suggestion that DISC1 could act as a key hub protein for processes of potential importance to mental health (Camargo et al., 2007).

A diverse number of identified functions collectively explain many aspects of the behavioral control attributed to DISC1. Most prominent are functions in neuronal migration (Kamiya et al., 2005), the integration of adult-born neurons (Duan et al., 2007), Wnt signaling (Mao et al., 2009), the development of dopaminergic neurotransmission (Niwa et al., 2010), NMDA neurotransmission (Hayashi-Takagi et al., 2010) and GABA neurotransmission (Kim et al., 2012). These functions are not mutually exclusive, but so far no unifying theory has been presented to explain which function is most salient, and in which context.

In view of this large number of interaction partners and functions, it has become customary to refer to DISC1 as a scaffold or scaffolding protein, for example, in recent reviews by ourselves and others (Brandon and Sawa, 2011; Bradshaw and Porteous, 2012; Korth, 2012; Thomson et al., 2013). Looking back on a decade of structure-function research on DISC1, we set out to investigate to what extent its classification as a conventional scaffold protein is indeed backed by experimental evidence, in order to help develop an overview of the roles of DISC1 in the cell and in the pathology of major mental illness.

The characteristics of a typical scaffold protein

In order to evaluate whether DISC1 qualifies as a *bona fide* scaffold protein, it is first important to review a few well-established examples of this group of proteins. Scaffold proteins are normally conceived as modulators of cellular pathways, conventionally lacking enzymatic activity and instead exerting their function by simultaneously interacting with multiple macromolecules, offering 'a simple, flexible strategy for regulating selectivity in pathways, shaping output behaviours, and achieving new responses from pre-existing signalling components' (Good et al., 2011). They usually act as specificity elements that selectively channel signaling between its bound partners (Zeke et al., 2009).

The concept of scaffolding proteins has been established for almost two decades (Choi et al., 1994; Zeke et al., 2009). Among the first scaffold proteins to be thoroughly investigated was Ste5 (Sterile 5), which was known to affect the activity of three enzymes from the *Saccharomyces cerevisiae* mitogen-activated protein kinase (MAPK) pathway (Ste11, Ste7, Fus3), and so was initially assumed to exist as an upstream signaling element (Hasson et al., 1994). Further research instead demonstrated Ste5 to be responsible for tethering these proteins and forming a multi-kinase complex (Choi et al., 1994). Thus, Ste5 effectively brings enzyme-substrate pairs of the cascade into physical proximity, resulting in more efficient signal transduction. Mathematical modeling studies revealed that the active phosphorylated intermediate species of Ste11, Ste7 and Fus3, when bound by the Ste5 scaffold protein, have a minimal probability of being consumed by competing signaling pathways or by non-specific phosphatases within the cell (Locasale et al., 2007). These studies also demonstrated that the signaling proteins bound to a scaffold protein will display restricted enzymatic activity due to their spatial arrangement. Finally, the ability of Ste5 to regulate the pathway was dependent on whether a positive (Ste50) or negative (Msg5) regulatory molecule was bound to it (Bashor et al., 2008).

A-kinase anchoring proteins (AKAPs) are another group of scaffold proteins, classified by their capacity to associate with protein kinase A (PKA) (Diviani and Scott, 2001). These proteins act to recruit signaling components to specific subcellular locations, for example AKAP350/450 targets PKA and protein kinase C to sites within the centrosome (Shanks et al., 2002), while in the heart a shorter splice variant of it, Yotiao, brings together PKA, PP1, PDE4D3 and the potassium ion channel and thereby locally regulates the β-adrenergic stimulation (Li et al., 2012).

Additional common properties of scaffolding proteins can be seen from other examples in the literature. For example, the mammalian Ste5 homologue Kinase Suppressor of Ras (KSR), depending on its phosphorylation state, mediates the translocation of multiple MAPK signaling components from the cytoplasm to the plasma membrane (Muller et al., 2001). Inactivation No Afterpotential D () is another scaffold protein expressed in the photoreceptor cells of *Drosophila* which localize and promote phosphorylation of transient receptor potential Ca^{2+} channels, thereby enhancing the quick adaptability of the visual system in fruit flies (Popescu et al., 2006). Homer 2 and Homer 3 are cytoplasmic scaffold proteins

that negatively regulate T-cell activation in mammals by binding to the Nuclear Factor of Activated T cells protein and shielding it from dephosphorylation. Homer-deficient mice developed autoimmune-like pathology, implying the importance of proper regulation of T-cell activation by the Homer family of scaffold proteins (Huang et al., 2008).

While the overall three-dimensional folds of scaffold proteins are quite diverse, our analysis indicates that the presence of coiled coil regions is a widespread feature. This super-secondary structure offers several advantages to the orchestration of multi-protein complexes (Rose and Meier, 2004). First and foremost, these include the ability to homo- or hetero-oligomerize, resulting in formation of supercoils involving different numbers of helices. Since coiled coil proteins often contain distinct functional domains on the same polypeptide chain, this oligomerization propensity provides a first level of clustering diverse (catalytic or non-catalytic) activities. In a few cases, formation of coiled coil dimers has been shown to be subject to regulation, such as by temperature or phosphorylation state (Hurme et al., 1996; Szilak et al., 1997). An additional level of complexity is usually added by different types of protein-protein interactions, typically with signaling proteins and enzymes, which may be mediated by the coiled coil domains themselves or by associated modules.

Second, scaffolding functions are typically assigned to elongated or rod-like molecules, and long helical supercoils provide an efficient means of accomplishing this geometry. In addition to providing an extended surface for protein-protein interactions, such elongated structures may serve as molecular rulers or spacers, precisely defining both the distances and relative orientation of associated molecules. An interesting variation to this theme is found in proteins of the spectrin superfamily. In this case, rod-like structures are formed from a series of so-called spectrin repeats, which are relatively short coiled coils sometimes connected by continuous helices (Djinovic-Carugo et al., 2002). Despite obvious structural constraints, spectrin repeats have evolved to accomplish a surprising variety of interactions also involving non-coiled coil proteins.

Finally, coiled coil domains are also commonly found in polypeptides determining the shapes and mechanical properties of cells and organelles, such as centrosomal proteins, golgins and motor proteins (Rose and Meier, 2004). Besides their structural roles (akin to scaffolds in human construction works), many of these proteins are also involved in signal transduction networks, and may thus qualify as scaffolding proteins in the sense used in this review.

Therefore, there are a number of features common to many scaffold proteins, namely that they:

- Bind to multiple protein components of the same signaling pathway, increasing both the efficiency and specificity of enzymatic cascades.
- Target the bound proteins to specific subcellular compartments, restricting a particular response to the appropriate location in the cell.
- Regulate the signaling pathway by enhancing or reducing the enzymatic activity of bound proteins.
- Shield the activated signaling intermediates preventing from any non-specific activity and consumption such as dephosphorylation (Locasale et al., 2007; Shaw and Filbert, 2009).
- Often have a high coiled coil-forming propensity, facilitating diverse and complex protein interactions.

With these criteria in mind, we will address each of these points in turn, and investigate how well DISC1 fits with them, based on published literature of its cellular functions.

Does DISC1 fit with these criteria for a classical scaffold protein?

Binding multiple proteins of the same signaling pathway

Among the many known protein-binding partners of DISC1, there are numerous examples of pairs of proteins that also exist in complexes with each other, particularly at the centrosome (reviewed in: Wang and Brandon, 2011; Bradshaw and Porteous, 2012), including the motor protein dynein and its associated proteins dynactin, Lissencephaly 1 (LIS1), Nuclear Distribution Element 1 (NDE1) and NDE-Like 1 (NDEL1) (Millar et al., 2003; Morris et al., 2003; Ozeki et al., 2003; Brandon et al., 2004) as well as the proteins Pericentriolar Material 1 (PCM1) and Bardet-Biedl Syndrome 4 (BBS4), which are together important for neuronal migration (Kamiya et al., 2008). DISC1 and NDEL1 have each been seen to interact with the mitochondrial protein Mitofilin (Park et al., 2010). Given the multiple distinct protein binding regions of DISC1 (reviewed in: Soares et al., 2011), it is highly likely that DISC1 is capable of binding to multiple different interaction partners simultaneously.

Evidence for such direct interactions can come from recombinant protein binding studies, once the individual proteins have been established to complex in a pairwise

fashion in the cell. For example, the kinesin motor protein KIF5A and adaptor protein Growth factor Receptor-Bound protein 2 (GrB2) interact *in vitro* in the presence, but not absence, of recombinant DISC1, strongly suggesting that the three proteins complex, through simultaneous binding to DISC1 (Shinoda et al., 2007). KIF5A-NDEL1-DISC1 ternary complexes have also been demonstrated in this way (Taya et al., 2007). In an analogous approach, use of DISC1 knockdown by shRNA reduced the co-immunoprecipitation of neurodevelopmental proteins Fasciculation and Elongation protein Zeta-1 (FEZ1) and NDEL1 from neuronal progenitor lysates (Kang et al., 2011), elegantly providing evidence for FEZ1-DISC1-NDEL1 complexes in an *in vivo* system. Conversely, over-expression of DISC1 enhances the interactions of both the microtubule proteins LIS1 and NDEL1 (Brandon et al., 2004) and of the synaptic proteins Kalirin-7 (Kal-7) and Postsynaptic Density protein 95 (PSD95) (Hayashi-Takagi et al., 2010), implying that these proteins co-complex at least in part through simultaneous interaction with DISC1.

At a minimum, DISC1 therefore appears to act as a scaffold for protein complexes involved in neurite outgrowth and function. While to date no published, confirmed examples of complexes involving both DISC1 and at least two enzymes from the same signaling pathway have been reported, candidate pathways do exist. Specifically, DISC1 binds Glycogen Synthase Kinase 3β (GSK3β, Mao et al., 2009), a key kinase of the Wnt signaling cascade, as well as two other modulators of this pathway, DIX Domain Containing 1 (Dixdc1, Singh et al., 2010) and Girdin (also known as KIAA1212, Enomoto et al., 2009; Kim et al., 2009). Similarly, DISC1 interacts with a range of phosphodiesterase 4 (PDE4) isoforms (Millar et al., 2005; Murdoch et al., 2007), which degrade cAMP as part of a negative feedback involving PKA. While DISC1 is not known to directly regulate PKA, it does interact with both Activating Transcription Factor 4 (ATF4) and NDE1 (Millar et al., 2003; Morris et al., 2003; Burdick et al., 2008; Sawamura et al., 2008; Bradshaw et al., 2009), two PKA-substrates each found in complex with PDE4B (Elefteriou et al., 2005; Bradshaw et al., 2008). Thus it is plausible that DISC1-GSK3β-regulator or DISC1-PDE4-substrate of PKA complexes could exist *in vivo*, fulfilling a key criterion for DISC1 having a scaffold protein function in GSK3β/PDE4B signaling. Interconnection between the PDE4 and GSK3 pathways has also been described (Carlyle et al., 2011; Lipina et al., 2012).

The ability of DISC1 to bind multiple proteins simultaneously is likely to be facilitated by its propensity to form oligomers, and thus potentially to present more protein binding domains at the same time. Of particular interest in this respect, a C-terminal DISC1 (640–854) fragment expressed in *Escherichia coli* was shown to interact with NDEL1 when the fragment was in an octameric form in a cell free assay (Leliveld et al., 2008), a finding that was later corroborated with full-length DISC1 (Narayanan et al., 2011). These findings suggest that the multimerization state of DISC1 may critically influence its protein interactions (Brandon et al., 2009).

Recruitment of proteins to specific cellular locations

As a putative scaffold protein, DISC1 would be expected to recruit a subset of its protein interaction partners to specific subcellular locations in order to facilitate their functions in a regulated manner. This appears to be the case with BBS4, which is recruited to the centrosome by DISC1 in a phosphorylation-dependent manner (Ishizuka et al., 2011). This in turn leads to the recruitment of further proteins, PCM1 and ninein, to the centrosome, from where DISC1, BBS4 and PCM1 are together implicated in regulating radial neuron migration (Kamiya et al., 2008). In a similar manner, DISC1 over-expression leads to increased expression of ATF4 within the nucleus (Pletnikov et al., 2007), where DISC1 and ATF4 have been shown to cumulatively repress cAMP Response Element-dependent gene transcription (Sawamura et al., 2008).

A better understood example of DISC1-driven recruitment is its role in bringing proteins to the axonal tips (Kamiya et al., 2006; Shinoda et al., 2007; Taya et al., 2007; Enomoto et al., 2009), which it appears to do by simultaneously interacting with kinesin motor complexes and the cargo proteins, facilitating their transport in an anterograde direction from the centrosome along the microtubule network (Shinoda et al., 2007; Taya et al., 2007).

Additionally, over-expression of DISC1 has been reported to recruit several of its interaction partners to punctate structures within the cytoplasm (Morris et al., 2003; Burdick et al., 2008; Wang et al., 2011), although whether this represents physiological recruitment or the known ability of DISC1 to bring other proteins into insoluble aggregates when highly concentrated (Ottis et al., 2011; Bader et al., 2012) remains unclear. We do not see the inherent aggregation propensity of DISC1 to be in contradiction to a possible function in scaffolding. As noted earlier, the multimerization-dependent interactions of DISC1 with NDEL1 (Leliveld et al., 2008) and the aggregation-dependent interactions with dysbindin or Collapsin Response Mediator Protein 1 are likely to be in a continuum of DISC1 multimer-dependent interactions as outlined in Brandon et al. (2009).

DISC1 therefore appears to play roles in protein recruitment, notably to the centrosome and via kinesin-related transport along axons, although its direct relevance to enzymes or signaling pathways is unclear, as summarized in Table 1.

Ability to modulate the enzymatic activity of binding partners

DISC1 interacts with multiple enzymes for which it is not a known substrate, implicating it as a regulator of their activity, as has been demonstrated in several cases. DISC1 binds to various members of the PDE4 family, which are responsible for the breakdown of cAMP, a second messenger for various stimuli and crucial in learning, memory and mood regulation (Millar et al., 2005). DISC1 holds PDE4 in an inactive state and can then release specific isoforms in response to cAMP-activated PKA (Murdoch et al., 2007). It was also shown that DISC1, via PDE4, regulates NDE1 phosphorylation by PKA, thereby modulating the organization of the NDE1-NDEL1-LIS1 complex (Bradshaw et al., 2011).

DISC1 has also been shown to repress the transcription-regulating activity of ATF4 (Sawamura et al., 2008). It appears to do this while ATF4 is present at its DNA target sites by simultaneously binding to ATF4 and recruiting the transcriptional repression factor Nuclear receptor Co-Repressor (N-CoR) (Sawamura et al., 2008), in a process that is regulated by both dopamine and PKA signaling (Soda et al., 2013). It is of interest that one of the genes directly affected by DISC1 and ATF4 is *PDE4D*, demonstrating the interconnectedness of pathways involving DISC1 (Soda et al., 2013).

DISC1 also inhibits GSK3β activity by direct physical interaction, preventing it from phosphorylating β-catenin, with important consequences for neural progenitor cell proliferation during cortical development (Mao et al., 2009). DISC1 co-modulates this pathway with its interaction partner Dixdc1. NDEL1 also exists in this complex, and during neuronal migration, phosphorylation of Dixdc1 by cyclin-dependent kinase 5 facilitates the Dixdc1-NDEL1 interaction (Singh et al., 2010).

GSK3β is also inhibited by Akt signaling, which in turn is regulated by DISC1 through the protein Girdin (Enomoto et al., 2009). DISC1 binds to Girdin, preventing it from activating Akt, a key kinase that regulates actin polymerization and cell motility (Enomoto et al., 2009). This function of DISC1 and the Akt-mTOR pathway has been shown to be important in regulating neuronal development in the dentate gyrus of the hippocampus (Kim et al., 2009).

Other examples of DISC1 affecting the enzymatic activity of proteins include the oligopeptidase activity of NDEL1, which is inhibited by DISC1 interaction (Hayashi et al., 2005). Interestingly, this NDEL1 enzyme activity is reduced in patients with schizophrenia, indicating the importance of regulation of NDEL1's activity for normal functioning of the brain (Gadelha et al., 2013). DISC1 also plays a crucial role in neuronal connectivity, via modulation of Ras-related C3 botulinum substrate 1 (Rac1) signaling via triple functional domain protein (TRIO) and Kal-7. DISC1 binds to TRIO, facilitating its Rac1 guanine nucleotide exchange factor ability through inhibition of its alternative Rho activating function (Chen et al., 2011) thereby promoting axon guidance. Conversely, DISC1 inhibits the alternative Rac1 guanine nucleotide exchange factor Kal-7 (Hayashi-Takagi et al., 2010). DISC1 also interacts with and inhibits Traf2 and Nck-interacting kinase (TNIK), leading to increased degradation of several key postsynaptic density proteins (Wang et al., 2011).

For a subset of its interaction partners, DISC1 therefore appears to be crucial for modulating the timing of their activity, normally through inhibition of catalytic functions. Thus DISC1 appears to be not merely a stationary sequester but an active regulator of the enzymatic behavior of its binding partners.

Shielding of activated signaling intermediates

There are a couple of indications showing that DISC1 may be able to shield specific signaling cascade intermediates

Table 1 A list of protein binding partners of DISC1 known to be recruited by DISC1 to distinct subcellular locations.

Subcellular region	Proteins
Centrosome	Bardet-Biedl syndrome 4, CAMDI, dynein IC, Lissencephaly 1, Ninein, p150^{glued} and Pericentriolar Material 1
Distal parts of the axon	14-3-3ε, Girdin, Growth factor Receptor-Bound protein 2, Lissencephaly 1 and NDE-Like 1
Nucleus	Activating Transcription Factor 4
Aggregates/aggresome	Collapsin Response Mediator Protein 1 and dysbindin
Cytoplasmic punctate structures	ATF5, Nuclear Distribution Element 1, NDE-Like 1 and Traf2 and Nck-interacting kinase

See main text for citations.

from undesired consumption. One example is serine racemase (SR), the enzyme responsible for production of *D*-serine, a co-agonist of the NMDA receptor. DISC1 was seen to complex with SR, with over-expression of a DISC1 mutant leading to an increased rate of ubiquitination and degradation of SR, but no down-regulation at the mRNA level (Ma et al., 2013). Thus, DISC1 binding is essential for the stability of SR, protecting it from degradation and facilitating the production of *D*-serine.

Similarly, the mitochondrial inner membrane protein Mitofilin was shown to bind to DISC1, with shRNA-targeted depletion of DISC1 or over-expression of a mutant leading to mitochondrial dysfunction (Park et al., 2010) resembling the effect of Mitofilin knockdown. Interestingly, over-expression of Mitofilin in DISC1-depleted cells restored these mitochondrial functions, strongly implying that DISC1 stabilizes it, a theory reinforced by experiments showing that the absence of DISC1 triggers ubiquitination of Mitofilin (Park et al., 2010).

These two examples indicate that DISC1 possesses the ability to shield some of its binding partners, with SR providing an example directly relevant to signaling pathways, as predicted for a scaffold protein.

Structural basis for DISC1 as a scaffold protein

The precise three-dimensional structure of DISC1 is still unknown and the protein shows no obvious sequence similarity to any entry in the Protein Data Bank, precluding conventional homology modeling. Previous analyses have indicated that the N-terminal region of DISC1 (330–350 residues) is likely to be largely disordered, with the remainder of the protein consisting of helical or coiled-coil domains (Soares et al., 2011). Additionally, two putative UVR domains, each containing a pair of antiparallel helices connected by a hairpin, have been predicted (Sanchez-Pulido and Ponting, 2011). A strategy to yield tentative three-dimensional fold information when obvious homologues with known structure are unavailable is given by threading approaches, which refers to a group of algorithms designed to detect faint similarities between a protein of interest and sequences with a known fold. In contrast to classical alignment strategies, sequences are not compared directly but represented as profiles, which can be expressed as position-specific scoring matrices or, in more sophisticated implementations, as hidden Markov models (Dunbrack, 2006). The method is based on the assumption that polypeptides exhibiting similar patterns of side chains tend to fold in a similar way. Such fold recognition by threading does not, however, imply any statement on the evolutionary relationship between the proteins involved.

We have subjected the DISC1 sequence to several current structure prediction pipelines, including Phyre^2 (Kelley and Sternberg, 2009), I-TASSER (Roy et al., 2010) and SAM-T08 (Karplus et al., 1998), which make use of different algorithms for the detection of potential templates. Out of the large number of profile-based alignments obtained, several long-range matches are particularly noteworthy (Figure 1A). In some of these, the majority of the C-terminal portion of DISC1 is mapped to extended helical structures, such as the spectrin repeat region of α-actinin, or the channel-forming colicin Ia. A second group of alignments indicate that large parts of the molecule may resemble the importin-β structure, a solenoid fold made up by lateral association of numerous HEAT repeats (containing two α-helices each), resulting in a spring-like superstructure. The predictive value of the resulting models, however, is questionable as the sequence profile of the DISC1 C-terminal domain appears to be equally compatible with very different arrangements of α-helices, making any prediction highly ambiguous. More importantly than this, critical assessment of model quality yields relatively low scores for all candidates. The situation can be slightly improved by manual adjustment of templates and alignments as well as additional energy minimization cycles, resulting in the model shown in Figure 1B, but the overall quality is still inferior to that expected of good homology models. These low-quality scores are mainly related to problems in side-chain packing and solvent accessibility: despite the apparent high confidence of profile matches, the distribution of hydrophobic and polar/charged side chains deviates from expectations. In general, extended hydrophobic patches observed on a protein surface may also represent sites of high-affinity protein-protein interactions, consistent with a scaffolding role. In the case of DISC1, however, it appears more likely that its C-terminal domain contains helical elements in an arrangement that is either not yet represented in the PDB or cannot be matched by the algorithms currently available.

Discussion

In this review we have analyzed the published literature on the DISC1 protein in comparison with the characteristics typical of scaffold proteins in order to determine how well DISC1 fits with that label (summarized in Table 2).

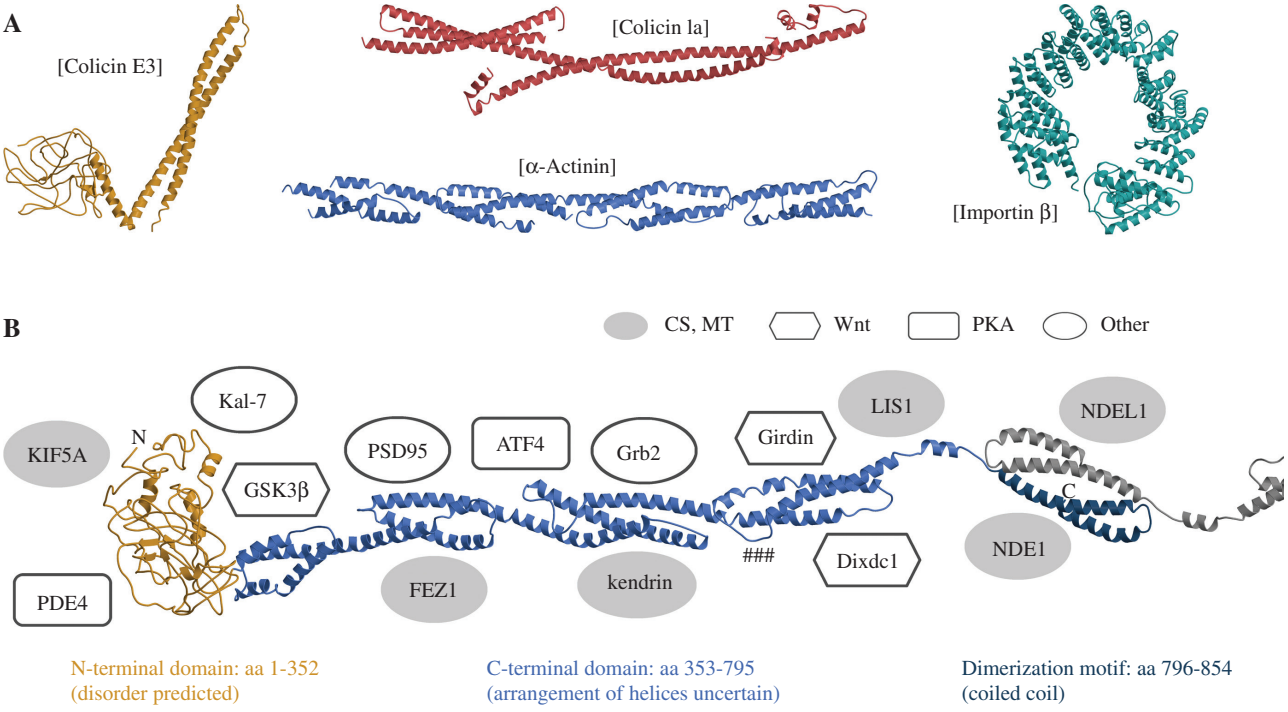

Figure 1 Speculative representations of possible structures of DISC1.
(A) Models derived from single templates (as indicated in square brackets), using profile-based alignments provided by Phyre[2] (for colicins and α-actinin) and I-TASSER (for importin β), respectively. Note the different arrangement of helices. (B) A speculative model of full-length DISC1 composed using MODELLER (Šali and Blundell, 1993) and modified versions of the Phyre[2] alignments. Specifically, amino acids 142–346, 351–773 and 782–829 were mapped to the N-terminal domain of colicin E3 (PDB code 1JCH, Soelaiman et al., 2001), the spectrin repeats of α-actinin (PDB code 1SJJ, Liu et al., 2004), and the coiled coil-domain of the transcription factor FOXP3 (PDB code 4I1L, Song et al., 2012), respectively. Regions without detectable profile matches (1–141 and 830–854) were built *ab initio* by POING (Jefferys et al., 2010), as implemented in Phyre[2]. The resulting model was subjected to an additional energy minimization in the YASARA force field (Krieger et al., 2009). Coloring indicates three major segments of the molecule, comprising residues 1–352 (yellow), 353–795 (blue), and 796–854 (dark blue), respectively. The C-terminus of the partner molecule in a putative DISC1 dimer is shown in gray. Please note that the dimerization motif is being defined here by the terminal coiled coil region predicted to commence around residue 800. In order to visualize the scaffolding function of DISC1, several established interaction partners are indicated (CS, centrosomal protein; MT, microtubular transport; Wnt, Wnt/Glycogen Synthase Kinase 3β pathway; PKA, PKA/PDE4 signaling). Hashes denote a region implicated in DISC1 oligomerization.

Table 2 A summary of typical functions of scaffold proteins and their relevance to DISC1.

Scaffolding feature	Examples of DISC1 exhibiting the feature
Binding multiple proteins of the same signaling pathway	– Forms ternary complexes with KIF5-Growth factor Receptor-Bound protein 2, KIF5A-Nuclear Distribution Element 1, Fasciculation and Elongation protein Zeta-1-NDE-Like 1 and Kal7-Postsynaptic Density protein 95 – Potential signaling-related complexes with Glycogen Synthase Kinase 3β, DIX Domain Containing 1 and Girdin and with PDE4B and substrates of protein kinase A
Recruitment of proteins to specific cellular locations	– Upon phosphorylation recruits Bardet-Biedl syndrome 4 proteins to the centrosome – Recruits Activating Transcription Factor 4 to the nucleus – Role in protein transport via Kinesin to the axon
Modulating the enzymatic activity of binding partners	– Binds inactive PDE4B, in a protein kinase A-dependent manner – Repress Activating Transcription Factor 4 activity – Inhibits the activity of Glycogen Synthase Kinase 3β, Nuclear Distribution Element-Like 1 and Traf2 and Nck Interacting Kinase. Blocks AKT-mTOR signaling pathway via Girdin
Shielding activated signaling pathway intermediates from inappropriate consumption	– Prevents ubiquitination of serine racemase – Prevents ubiquitination of Mitofilin

From these data, it is clear that DISC1 exhibits certain aspects of a scaffold protein but contrasts with classical scaffold proteins, such as Ste5, which typically channel a single signal cascade by binding to multiple components of it. Instead DISC1 appears to be involved in the fine-tuning of a large number of inter-related signaling pathways with links to neuronal development and function (Figure 2). Strikingly there are no established examples, to the best of our knowledge, of DISC1 directly enhancing the activity of an enzyme through recruitment of substrates and co-factors or through anchoring the enzyme itself to the location of action, as would be predicted for scaffold proteins. Instead, the principal regulatory activity of DISC1 appears to be the inhibition of enzymes through direct physical contact, as is the case for Kal-7, GSK3β, PDE4, TNIK and the oligopeptidase activity of NDEL1 (Hayashi et al., 2005; Millar et al., 2005; Murdoch et al., 2007; Mao et al., 2009; Hayashi-Takagi et al., 2010; Wang et al., 2011) or through the recruitment of repressive cofactors, such as N-CoR to ATF4 (Sawamura et al., 2008). The partial exceptions are, firstly, SR which is stabilized through interaction with DISC1 (Ma et al., 2013), leading to a net increase in its activity and, secondly, that DISC1 can enhance the ability of TRIO to act as an exchange factor for Rac1, through the binding of DISC1 to a second active enzymatic site on TRIO, inhibiting this latter activity and favouring Rac1 as a substrate (Chen et al., 2011).

If DISC1 is to regulate such a diverse array of processes, it is also likely to be itself tightly regulated. In support of this, multiple PKA phosphorylation sites on DISC1 have been described which act as switches between Wnt-signaling, centrosome-related functions (Ishizuka et al., 2011) and regulation of ATF4-based transcriptional activity, respectively (Soda et al., 2013). Complex regulation of DISC1 expression is also suggested by the existence of over 40 splice variants of *DISC1* in the brain (Nakata

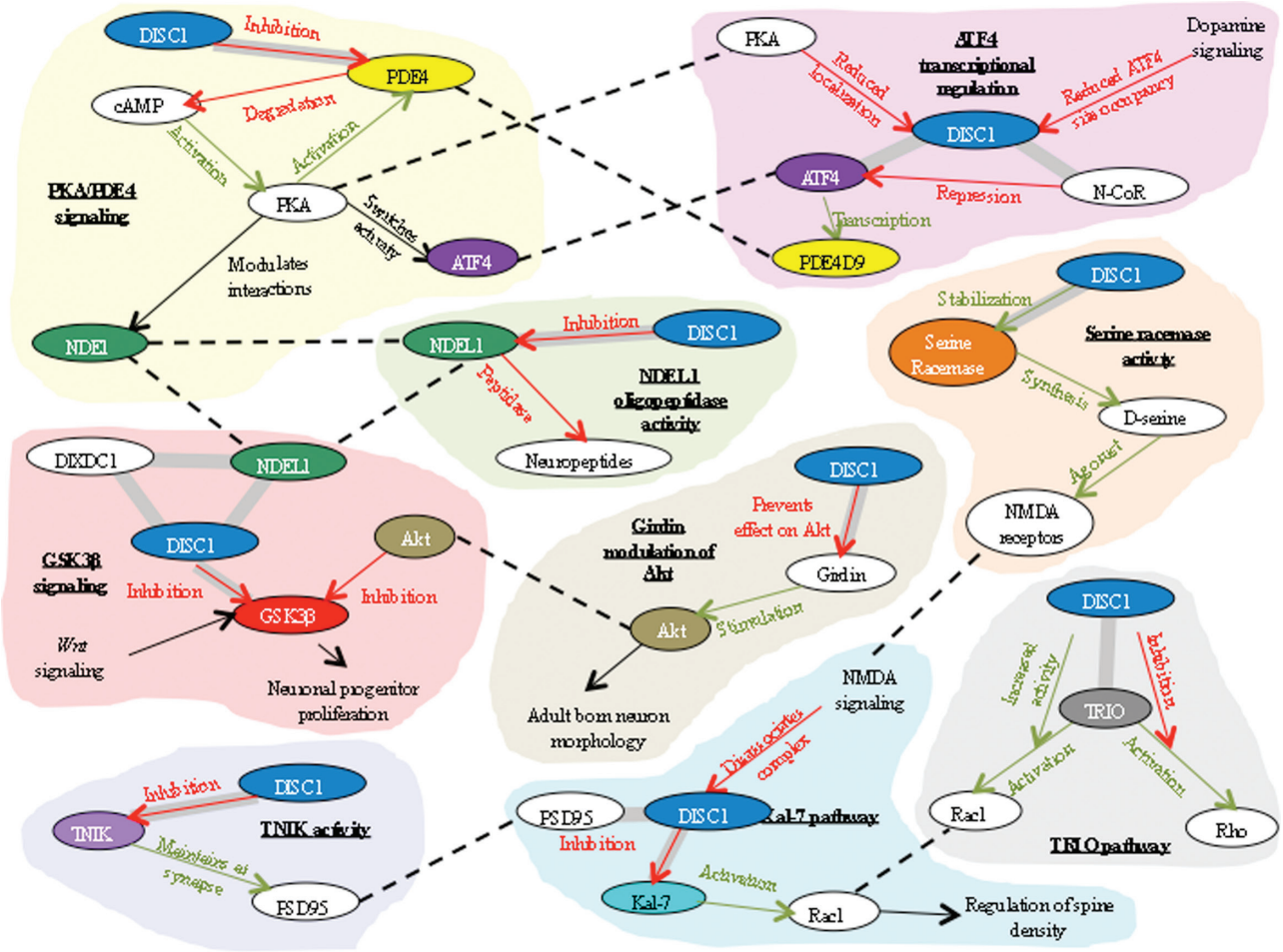

Figure 2 A schematic summary of nine interlinked signaling pathways in which DISC1 has been implicated.
Thick gray lines indicate direct protein interactions, arrows indicate interaction in the form of activation/stimulation (green), inhibition (red) or less easily defined effects (black). Identical or similar proteins found in multiple pathways are linked by hashed lines.

et al., 2009), some of which have been demonstrated to show altered protein-protein interactions (Newburn et al., 2011). This would allow for the existence of DISC1 species capable of only a subset of the functions of the full-length protein.

Another important factor likely to affect the scaffold-like functions of DISC1 is its multimerization. NDEL1 has been shown to interact with octameric, and depending on the assay used potentially dimeric, forms of DISC1, but not with higher-order oligomers (Leliveld et al., 2008; Narayanan et al., 2011), indicating that the interactions exhibit conformational specificity along with domain specificity. This also raises the obvious possibility of oligomer-specific interactions making DISC1 a multi-potent scaffold protein.

Parallels can be drawn between DISC1 and the known functions of the AKAP scaffold proteins. For example, like DISC1, AKAP9 has a complex pattern of alternate splicing (Welch et al., 2010) and like DISC1, its encoded proteins are involved in recruiting proteins to the centrosome (Shanks et al., 2002) among other locations, and can also inhibit a subset of their interaction partners, such as adenylate cyclases (Piggott et al., 2008). More generally, AKAPs form multivalent signaling complexes involving a large array of binding partners (Welch et al., 2010) and have been described as acting as the nucleus of a 'transduceosome' for a set of related signaling processes (Feliciello et al., 2001). DISC1 appears to be acting in a manner at least partially analogous to this; however, while AKAPs focus on the anchoring of the PKA protein and its substrates and associated molecules, DISC1 appears to instead form multiple seemingly distinct signaling complexes involving a wide array of signaling enzymes.

In conclusion, DISC1 possesses certain properties of a scaffold protein, but in a unique way. As it stands as a complex regulator for various interrelated pathways in neurodevelopment, unlike the classical single pathway scaffolds, it is very crucial for normal brain functioning. Further investigation of these DISC1-related pathways individually, considering its scaffolding nature, would likely contribute to a better understanding of DISC1's mechanism. Thus, in addition to being 'Disrupted in Schizophrenia', DISC1 also appears to be a 'Diverse Inhibitor of Signaling Complexes', and it is likely that modulation of this activity, for example by specific blocking of its interaction sites, would form the most promising lead for any future DISC1-inspired therapeutic intervention.

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References

Bader, V., Tomppo, L., Trossbach, S.V., Bradshaw, N.J., Prikulis, I., Leliveld, S.R., Lin, C.-Y., Ishizuka, K., Sawa, A., Ramos, A., et al. (2012). Proteomic, genomic and translational approaches identify CRMP1 for a role in schizophrenia and its underlying traits. Hum. Mol. Genet. *21*, 4406–4418.

Bashor, C.J., Helman, N.C., Yan, S., and Lim, W.A. (2008). Using engineered scaffold interactions to reshape MAP kinase pathway signaling dynamics. Science *319*, 1539–1543.

Blackwood, D.H.R., Fordyce, A., Walker, M.T., St. Clair, D.M., Porteous, D.J., and Muir, W.J. (2001). Schizophrenia and affective disorders – cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: Clinical and P300 findings in a family. Am. J. Hum. Genet. *69*, 428–433.

Bradshaw, N.J., Christie, S., Soares, D.C., Carlyle, B.C., Porteous, D.J., and Millar, J.K. (2009). NDE1 and NDEL1: Multimerisation, alternate splicing and DISC1 interaction. Neurosci. Lett. *449*, 228–233.

Bradshaw, N.J., Ogawa, F., Antolin-Fontes, B., Chubb, J.E., Carlyle, B.C., Christie, S., Claessens, A., Porteous, D.J., and Millar, J.K. (2008). DISC1, PDE4B, and NDE1 at the centrosome and synapse. Biochem. Biophys. Res. Commun. *377*, 1091–1096.

Bradshaw, N.J. and Porteous, D.J. (2012). DISC1-binding proteins in neural development, signalling and schizophrenia. Neuropharmacology *62*, 1230–1241.

Bradshaw, N.J., Soares, D.C., Carlyle, B.C., Ogawa, F., Davidson-Smith, H., Christie, S., Mackie, S., Thomson, P.A., Porteous, D.J., and Millar, J.K. (2011). PKA phosphorylation of NDE1 is DISC1/PDE4-dependant and modulates its interaction with LIS1 and NDEL1. J. Neurosci. *31*, 9043–9054.

Brandon, N.J., Handford, E.J., Schurov, I., Rain, J.-C., Pelling, M., Duran-Jimeriz, B., Camargo, L.M., Oliver, K.R., Beher, D., Shearman, M.S., et al. (2004). Disrupted in Schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: implications for schizophrenia and other major neurological disorders. Mol. Cell Neurosci. *25*, 42–55.

Brandon, N.J., Millar, J.K., Korth, C., Sive, H., Singh, K.K., and Sawa, A. (2009). Understanding the role of DISC1 in psychiatric disease and during normal development. J. Neurosci. *29*, 12768–12775.

Brandon, N.J. and Sawa, A. (2011). Linking neurodevelopmental and synaptic theories of mental illness through DISC1. Nat. Rev. Neurosci. *12*, 707–722.

Burdick, K.E., Kamiya, A., Hodgkinson, C.A., Lencz, T., DeRosse, P., Ishizuka, K., Elashvili, S., Arai, H., Goldman, D., Sawa, A., et al. (2008). Elucidating the relationship between *DISC1*, *NDEL1*, and *NDE1* and the risk for schizophrenia: evidence of epistasis and competitive binding. Hum. Mol. Genet. *17*, 2462–2473.

Callicott, J.H., Straub, R.E., Pezawas, L., Egan, M.F., Mattay, V.S., Hariri, A.R., Verchinski, B.A., Meyer-Lindenberg, A., Balkissoon, R., Kolachana, B., et al. (2005). Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. Proc. Natl. Acad. Sci. USA *102*, 8627–8632.

Camargo, L.M., Collura, V., Rain, J.-C., Mizuguchi, K., Hermjakob, H., Kerrien, S., Bonnert, T.P., Whiting, P.J., and Brandon, N.J. (2007). Disrupted in Schizophrenia 1 Interactome: evidence for the close connectivity of risk genes and a potential synaptic basis for schizophrenia. Mol. Psychiatry *12*, 74–86.

Carlyle, B.C., Mackie, S., Christie, S., Millar, J.K., and Porteous, D.J. (2011). Co-ordinated action of DISC1, PDE4B and GSK3b in modulation of cAMP signalling. Mol. Psychiatry *16*, 693–694.

Chen, S.Y., Huang, P.H., and Cheng, H.J. (2011). Disrupted-in-Schizophrenia 1-mediated axon guidance involves TRIO-RAC-PAK small GTPase pathway signaling. Proc. Natl. Acad. Sci. USA *108*, 5861–5866.

Choi, K.Y., Satterberg, B., Lyons, D.M., and Elion, E.A. (1994). Ste5 tethers multiple protein kinases in the MAP kinase cascade required for mating in S. cerevisiae. Cell *78*, 499–512.

Chubb, J.E., Bradshaw, N.J., Soares, D.C., Porteous, D.J., and Millar, J.K. (2008). The *DISC* locus in psychiatric illness. Mol. Psychiatry *13*, 36–64.

Diviani, D., and Scott, J.D. (2001). AKAP signaling complexes at the cytoskeleton. J. Cell Sci. *114*, 1431–1437.

Djinovic-Carugo, K., Gautel, M., Ylanne, J., and Young, P. (2002). The spectrin repeat: a structural platform for cytoskeletal protein assemblies. FEBS Lett. *513*, 119–123.

Duan, X., Chang, J.H., Ge, S., Faulkner, R.L., Kim, J.Y., Kitabatake, Y., Liu, X.-b., Yang, C.-H., Jordan, J.D., Ma, D.K., et al. (2007). Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. Cell *130*, 1146–1158.

Dunbrack, R.L., Jr. (2006). Sequence comparison and protein structure prediction. Curr. Opin. Struct. Biol. *16*, 374–384.

Elefteriou, F., Ahn, J.D., Takeda, S., Starbuck, M., Yang, X., Liu, X., Kondo, H., Richards, W.G., Bannon, T.W., Noda, M., et al. (2005). Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature *434*, 514–520.

Enomoto, A., Asai, N., Namba, T., Wang, Y., Kato, T., Tanaka, M., Tatsumi, H., Taya, S., Tsuboi, D., Kuroda, K., et al. (2009). Roles of disrupted-in-schizophrenia 1-interacting protein girdin in postnatal development of the dentate gyrus. Neuron *63*, 774–787.

Feliciello, A., Gottesman, M.E., and Avvedimento, E.V. (2001). The biological functions of A-kinase anchor proteins. J. Mol. Biol. *308*, 99–114.

Gadelha, A., Machado, M.F.M., Yonamine, C.M., Sato, J.R., Juliano, M.A., Oliveira, V., Bressan, R.A., and Hayashi, M.A.F. (2013). Plasma Ndel1 enzyme activity is reduced in patients with schizophrenia – A potential biomarker? J. Psychiatr. Res. *47*, 657–663.

Good, M.C., Zalatan, J.G., and Lim, W.A. (2011). Scaffold proteins: hubs for controlling the flow of cellular information. Science *332*, 680–686.

Hasson, M.S., Blinder, D., Thorner, J., and Jenness, D.D. (1994). Mutational activation of the STE5 gene product bypasses the requirement for G protein β and γ subunits in the yeast pheromone response pathway. Mol. Cell Biol. *14*, 1054–1065.

Hayashi-Takagi, A., Takaki, M., Graziane, N., Seshadri, S., Murdoch, H., Dunlop, A.J., Makino, Y., Seshadri, A.J., Ishizuka, K., Srivastava, D.P., et al. (2010). Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1. Nat. Neurosci. *13*, 327–332.

Hayashi, M.A.F., Portaro, F.C.V., Bastos, M.F., Guerreiro, J.R., Oliveira, V., Gorrao, S.S., Tambourgi, D.V., Sant'Anna, O.A., Whiting, P.J., Camargo, L.M., et al. (2005). Inhibition of NUDEL (nuclear distribution element-like)-oligopeptidase activity by disrupted-in-schizophrenia 1. Proc. Natl. Acad. Sci. USA *102*, 3828–3833.

Huang, G.N., Huso, D.L., Bouyain, S., Tu, J., McCorkell, K.A., May, M.J., Zhu, Y., Lutz, M., Collins, S., Dehoff, M., et al. (2008). NFAT binding and regulation of T cell activation by the cytoplasmic scaffolding Homer proteins. Science *319*, 476–481.

Hurme, R., Berndt, K.D., Namork, E., and Rhen, M. (1996). Additions and Corrections to DNA binding exerted by a bacterial gene regulator with an extensive coiled-coil domain. J. Biol. Chem. *271*, 17547.

Ishizuka, K., Kamiya, A., Oh, E.C., Kanki, H., Seshadri, S., Robinson, J.F., Murdoch, H., Dunlop, A.J., Kubo, K.-i., Furukori, K., et al. (2011). DISC1-dependent switch from progenitor proliferation to migration in the developing cortex. Nature *473*, 92–96.

Jefferys, B.R., Kelley, L.A., and Sternberg, M.J.E. (2010). Protein folding requires crowd control in a simulated cell. J. Mol. Biol. *397*, 1329–1338.

Kamiya, A., Kubo, K.-i., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., Sawamura, N., Park, U., Kudo, C., Okawa, M., et al. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. Nat. Cell Biol. *7*, 1167–1178.

Kamiya, A., Tan, P.L., Kubo, K., Engelhard, C., Ishizuka, K., Kubo, A., Tsukita, S., Pulver, A.E., Nakajima, K., Cascella, N.G., et al. (2008). Recruitment of PCM1 to the centrosome by the cooperative action of DISC1 and BBS4: a candidate for psychiatric illnesses. Arch. Gen. Psychiatry *65*, 996–1006.

Kamiya, A., Tomoda, T., Chang, J., Takaki, M., Zhan, C., Morita, M., Cascio, M.B., Elashvili, S., Koizumi, H., Takanezawa, Y., et al. (2006). DISC1-NDEL1/NUDEL protein interaction, an essential component for neurite outgrowth, is modulated by genetic variations of DISC1. Hum. Mol. Genet. *15*, 3313–3323.

Kang, E., Burdick, Katherine E., Kim, Ju Y., Duan, X., Guo, Junjie U., Sailor, Kurt A., Jung, D.-E., Ganesan, S., Choi, S., Pradhan, D., et al. (2011). Interaction between FEZ1 and DISC1 in regulation of neuronal development and risk for schizophrenia. Neuron *72*, 559–571.

Karplus, K., Barrett, C., and Hughey, R. (1998). Hidden Markov models for detecting remote protein homologies. Bioinformatics *14*, 846–856.

Kelley, L.A. and Sternberg, M.J. (2009). Protein structure prediction on the Web: a case study using the Phyre server. Nat. Protoc. *4*, 363–371.

Kim, J.Y., Duan, X., Liu, C.Y., Jang, M.H., Guo, J.U., Pow-anpongkul, N., Kang, E., Song, H., and Ming, G.L. (2009). DISC1 regulates new neuron development in the adult brain via modulation of AKT-mTOR signaling through KIAA1212. Neuron *63*, 761–773.

Kim, J.Y., Liu, C.Y., Zhang, F., Duan, X., Wen, Z., Song, J., Feighery, E., Lu, B., Rujescu, D., Clair, D.S., et al. (2012). Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. Cell *148*, 1051–1064.

Korth, C. (2012). Aggregated proteins in schizophrenia and other chronic mental diseases: DISC1opathies. Prion *6*, 1–8.

Krieger, E., Joo, K., Lee, J., Lee, J., Raman, S., Thompson, J., Tyka, M., Baker, D., and Karplus, K. (2009). Improving physical realism, stereochemistry, and side-chain accuracy in homology modeling: four approaches that performed well in CASP8. Proteins *77*, 114–122.

Kuroda, K., Yamada, S., Tanaka, M., Iizuka, M., Yano, H., Mori, D., Tsuboi, D., Nishioka, T., Namba, T., Iizuka, Y., et al. (2011). Behavioral alterations associated with targeted disruption of exons 2 and 3 of the Disc1 gene in the mouse. Hum. Mol. Genet. *20*, 4666–4683.

Leliveld, S.R., Bader, V., Hendriks, P., Prikulis, I., Sajnani, G., Requena, J.R., and Korth, C. (2008). Insolubility of Disrupted-in-Schizophrenia 1 disrupts oligomer-dependent interactions with Nuclear Distribution Element 1 and is associated with sporadic mental disease. J. Neurosci. *28*, 3839–3845.

Li, Y., Chen, L., Kass, R.S., and Dessauer, C.W. (2012). The A-kinase anchoring protein yotiao facilitates complex formation between adenylyl cyclase type 9 and the IKs potassium channel in heart. J. Biol. Chem. *287*, 29815–29824.

Lipina, T.V., Wang, M., Liu, F., and Roder, J.C. (2012). Synergistic interactions between PDE4B and GSK-3: DISC1 mutant mice. Neuropharmacology *62*, 1252–1262.

Liu, J., Taylor, D.W., and Taylor, K.A. (2004). A 3-D reconstruction of smooth muscle α-actinin by CryoEM reveals two different conformations at the actin-binding region. J. Mol. Biol. *338*, 115–125.

Locasale, J.W., Shaw, A.S., and Chakraborty, A.K. (2007). Scaffold proteins confer diverse regulatory properties to protein kinase cascades. Proc. Natl. Acad. Sci. USA *104*, 13307–13312.

Ma, T.M., Abazyan, S., Abazyan, B., Nomura, J., Yang, C., Seshadri, S., Sawa, A., Snyder, S.H., and Pletnikov, M.V. (2013). Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. Mol. Psychiatry *18*, 557–567.

Mao, Y., Ge, X., Frank, C.L., Madison, J.M., Koehler, A.N., Doud, M.K., Tassa, C., Berry, E.M., Soda, T., Singh, K.K., et al. (2009). Disrupted in Schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3b/b-Catenin signaling. Cell *136*, 1017–1031.

Millar, J.K., Christie, S., and Porteous, D.J. (2003). Yeast two-hybrid screens implicate DISC1 in brain development and function. Biochem. Biophys. Res. Commun. *311*, 1019–1025.

Millar, J.K., Pickard, B.S., Mackie, S., James, R., Christie, S., Buchanan, S.R., Malloy, M.P., Chubb, J.E., Huston, E., Baille, G.S., et al. (2005). DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signalling. Science *310*, 1187–1191.

Millar, J.K., Wilson-Annan, J.C., Anderson, S., Christie, S., Taylor, M.S., Semple, C.A.M., Devon, R.S., St Clair, D.M., Muir, W.J., Blackwood, D.H.R., et al. (2000). Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum. Mol. Genet. *9*, 1415–1425.

Miyoshi, K., Honda, A., Baba, K., Taniguchi, M., Oono, K., Fujita, T., Kuroda, S., Katayama, T., and Tohyama, M. (2003). *Disrupted-in-Schizophrenia 1*, a candidate gene for schizophrenia, participates in neurite outgrowth. Mol. Psychiatry *8*, 685–694.

Morris, J.A., Kandpal, G., Ma, L., and Austin, C.P. (2003). DISC1 (Disrupted-In-Schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5 and NUDEL: regulation and loss of interaction with mutation. Hum. Mol. Genet. *12*, 1591–1608.

Muller, J., Ory, S., Copeland, T., Piwnica-Worms, H., and Morrison, D.K. (2001). C-TAK1 regulates Ras signaling by phosphorylating the MAPK scaffold, KSR1. Mol. Cell *8*, 983–993.

Murdoch, H., Mackie, S., Collins, D.M., Hill, E.V., Bolger, G.B., Klussmann, E., Porteous, D.J., Millar, J.K., and Houslay, M.D. (2007). Isoform-selective susceptibility of DISC1/Phosphodiesterase-4 complexes to dissociation by elevated intracellular cAMP levels. J. Neurosci. *27*, 9513–9524.

Nakata, K., Lipska, B.K., Hyde, T.M., Ye, T., Newburn, E.N., Morita, Y., Vakkalanka, R., Barenboim, M., Sei, Y., Weinberger, D.R., et al. (2009). *DISC1* splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc. Natl. Acad. Sci. USA *106*, 15873–15878.

Narayanan, S., Arthanari, H., Wolfe, M.S., and Wagner, G. (2011). Molecular characterization of Disrupted in Schizophrenia-1 risk variant S704C reveals the formation of altered oligomeric assembly. J. Biol. Chem. *286*, 44266–44276.

Newburn, E.N., Hyde, T.M., Ye, T., Morita, Y., Weinberger, D.R., Kleinman, J.E., and Lipska, B.K. (2011). Interactions of human truncated DISC1 proteins: implications for schizophrenia. Transl. Psychiatry *1*, e30.

Niwa, M., Kamiya, A., Murai, R., Kubo, K.-i., Gruber, A.J., Tomita, K., Lu, L., Tomisato, S., Jaaro-Peled, H., Seshadri, S., et al. (2010). Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. Neuron *65*, 480–489.

Ottis, P., Bader, V., Trossbach, S.V., Kretzschmar, H., Michel, M., Leliveld, S.R., and Korth, C. (2011). Convergence of two independent mental disease genes on the protein level: Recruitment of dysbindin to cell-invasive DISC1 aggresomes. Biol. Psychiatry *70*, 604–610.

Ozeki, Y., Tomoda, T., Kleiderlein, J., Kamiya, A., Bord, L., Fujii, K., Okawa, M., Yamada, N., Hatten, M.E., Snyder, S.H., et al. (2003). Disrupted-in-Schizophrenia-1 (DISC-1): mutant truncation prevents binding to NudE-like (NUDEL) and inhibits neurite outgrowth. Proc. Natl. Acad. Sci. USA *100*, 289–294.

Park, Y.-U., Jeong, J., Lee, H., Mun, J.Y., Kim, J.-H., Lee, J.S., Nguyen, M.D., Han, S.S., Suh, P.-G., and Park, S.K. (2010). *Disrupted-in-schizophrenia* 1 (*DISC1*) plays essential roles in mitochondria in collaboration with Mitofilin. Proc. Natl. Acad. Sci. USA *107*, 17785–17790.

Piggott, L.A., Bauman, A.L., Scott, J.D., and Dessauer, C.W. (2008). The A-kinase anchoring protein Yotiao binds and regulates adenylyl cyclase in brain. Proc. Natl. Acad. Sci. USA *105*, 13835–13840.

Pletnikov, M.V., Xu, Y., Ovanesov, M.V., Kamiya, A., Sawa, A., and Ross, C.A. (2007). PC12 cell model of inducible expression of mutant DISC1: new evidence for a dominant-negative mechanism of abnormal neuronal differentiation. Neurosci. Res. *58*, 234–244.

Popescu, D.C., Ham, A.J., and Shieh, B.H. (2006). Scaffolding protein INAD regulates deactivation of vision by promoting phosphorylation of transient receptor potential by eye protein kinase C in *Drosophila*. J. Neurosci. *26*, 8570–8577.

Rose, A. and Meier, I. (2004). Scaffolds, levers, rods and springs: diverse cellular functions of long coiled-coil proteins. Cell Mol. Life Sci. *61*, 1996–2009.

Roy, A., Kucukural, A., and Zhang, Y. (2010). I-TASSER: a unified platform for automated protein structure and function prediction. Nat. Protoc. *5*, 725–738.

Šali, A. and Blundell, T.L. (1993). Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol *234*, 779–815.

Sanchez-Pulido, L. and Ponting, C.P. (2011). Structure and evolutionary history of DISC1. Hum. Mol. Genet. *20*, R175–R181.

Sawamura, N., Ando, T., Maruyama, Y., Fujimuro, M., Mochizuki, H., Honjo, K., Shimoda, M., Toda, H., Sawamura-Yamamoto, T., Makuch, L.A., et al. (2008). Nuclear DISC1 regulates CRE-mediated gene transcription and sleep homeostasis in the fruit fly. Mol. Psychiatry *13*, 1138–1148.

Shanks, R.A., Steadman, B.T., Schmidt, P.H., and Goldenring, J.R. (2002). AKAP350 at the Golgi apparatus. I. Identification of a distinct Golgi apparatus targeting motif in AKAP350. J. Biol. Chem. *277*, 40967–40972.

Shaw, A.S. and Filbert, E.L. (2009). Scaffold proteins and immune-cell signalling. Nat. Rev. Immunol. *9*, 47–56.

Shen, S., Lang, B., Nakamoto, C., Zhang, F., Pu, J., Kuan, S.-L., Chatzi, C., He, S., Mackie, I., Brandon, N.J., et al. (2008). Schizophrenia-related neural and behavioral phenotypes in transgenic mice expressing truncated Disc1. J. Neurosci. *28*, 10893–10904.

Shinoda, T., Taya, S., Tsuboi, D., Hikita, T., Matsuzawa, R., Kuroda, S., Iwamatsu, A., and Kaibuchi, K. (2007). DISC1 regulates Neurotrophin-induced axon elongation via interaction with Grb2. J. Neurosci. *27*, 4–14.

Singh, K.K., Ge, X., Mao, Y., Drane, L., Meletis, K., Samuels, B.A., and Tsai, L.-H. (2010). Dixdc1 is a critical regulator of DISC1 and embryonic cortical development. Neuron *67*, 33–48.

Soares, D.C., Carlyle, B.C., Bradshaw, N.J., and Porteous, D.J. (2011). DISC1: structure, function, and therapeutic potential for major mental illness. ACS Chem. Neurosci. *2* 609–632.

Soda, T., Frank, C., Ishizuka, K., Baccarella, A., Park, Y.-U., Flood, Z., Park, S.K., Sawa, A., and Tsai, L.-H. (2013). DISC1-ATF4 transcriptional repression complex: dual regulation of the cAMP-PDE4 cascade by DISC1. Mol Psychiatry doi:10.1038/mp.2013.38.

Soelaiman, S., Jakes, K., Wu, N., Li, C., and Shoham, M. (2001). Crystal structure of colicin E3: implications for cell entry and ribosome inactivation. Mol. Cell *8*, 1053–1062.

Song, X., Li, B., Xiao, Y., Chen, C., Wang, Q., Liu, Y., Berezov, A., Xu, C., Gao, Y., Li, Z., et al. (2012). Structural and biological features of FOXP3 dimerization relevant to regulatory T cell function. Cell Rep. *1*, 665–675.

Szilak, L., Moitra, J., Krylov, D., and Vinson, C. (1997). Phosphorylation destabilizes α-helices. Nat. Struct. Biol. *4*, 112–114.

Taya, S., Shinoda, T., Tsuboi, D., Asaki, J., Nagai, K., Hikita, T., Kuroda, S., Kuroda, K., Shimizu, M., Hirotsune, S., et al. (2007). DISC1 regulates the transport of the NUDEL/LIS1/14-3-3epsilon complex through kinesin-1. J. Neurosci. *27*, 15–26.

Thomson, P.A., Harris, S.E., Starr, J.M., Whalley, L.J., Porteous, D.J., and Deary, I.J. (2005). Association between genotype at an exonic SNP in DISC1 and normal cognitive aging. Neurosci. Lett. *389*, 41–45.

Thomson, P.A., Malavasi, E.L.V., Grünewald, E., Soares, D.C., Borkowska, M., and Millar, J.K. (2013). DISC1 genetics, biology and psychiatric illness. Front. Biol. *8*, 1–31.

Wang, Q. and Brandon, N.J. (2011). Regulation of the cytoskeleton by Disrupted-in-Schizophrenia 1 (DISC1). Mol. Cell Neurosci. *48*, 359–364.

Wang, Q., Charych, E.I., Pulito, V.L., Lee, J.B., Graziane, N.M., Crozier, R.A., Revilla-Sanchez, R., Kelly, M.P., Dunlop, A.J., Murdoch, H., et al. (2011). The psychiatric disease risk factors DISC1 and TNIK interact to regulate synapse composition and function. Mol. Psychiatry *16*, 1006–1023.

Welch, E.J., Jones, B.W., and Scott, J.D. (2010). Networking with AKAPs: context-dependent regulation of anchored enzymes. Mol. Interv. *10*, 86–97.

Zeke, A., Lukacs, M., Lim, W.A., and Remenyi, A. (2009). Scaffolds: interaction platforms for cellular signalling circuits. Trends. Cell Biol. *19*, 364–374.

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