How DISC1 Regulates Postnatal Brain Development: Girdin Gets In on the AKT

David Porteous1, * and Kirsty Millar1, *

1 Medical Genetics Section, Molecular Medicine Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh, EH4 2XU, Edinburgh, UK
2 Correspondence: david.porteous@ed.ac.uk (D.P.), kirsty.millar@ed.ac.uk (K.M.)
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In this issue of Neuron, Kim et al. and Enomoto et al. show that DISC1 plays a key role in regulating postnatal brain development though interaction with Girdin. Girdin in turn regulates AKT signaling. Thus, another facet of the role of DISC1 is established, shedding more light on fundamental brain processes and the developmental basis of major psychiatric disorders.

DISC1 in Schizophrenia, Protein-Protein Interaction, and Neurodevelopment

DISC1 was discovered as a novel gene at the breakpoint of a balanced (1;11) translocation that segregates with schizophrenia, bipolar disorder, and recurrent major depression in a large Scottish family (reviewed in Chubb et al., 2008). This discovery was both surprising and insightful. Here was a single genetic event that could give rise, with high penetrance (the majority of carriers are affected), to a range of psychiatric disorders that transcend traditional diagnostic boundaries, challenging many strongly held precepts about the genetic etiology of psychotic and mood disorders. Subsequent genetic studies have found supporting evidence for DISC1 as a generalized risk factor in a significant proportion of cases and have extended the diagnostic boundaries of influence to include autism spectrum disorder and cognitive aging (reviewed in Chubb et al., 2008).

Upon discovery, and showing no obvious similarity to other known genes or proteins, the function of DISC1 was far from clear. De novo biochemical and expression studies established that DISC1 was a multifunctional, neurodevelopmentally regulated scaffold protein, which when mutated in the mouse gave rise to developmental, behavioral, and pharmacological phenotypes that modeled important aspects of the human conditions (Clapcote et al., 2007; reviewed in Chubb et al., 2008). This scaffold function resolves the genetics paradox—the multiple proteins shown to interact with DISC1 are enriched for proteins known to have a role in neurodevelopment, neurosignaling, cytoskeletal and centromeric function, and the synapse, thus having the potential to simultaneously affect a wide range of plausible risk processes in susceptibility. And indeed, several of these DISC1 interactors turn out to be codependent or independent genetic risk factors (reviewed in Chubb et al., 2008).

The question now becomes: which of these many potential interactors matter most, and at what point in development and at what location in the brain? Previously, Duan et al. (2007) used an elegant, retrovirally mediated, single-cell RNAi strategy to selectively suppress mouse Disc1 expression in vivo in differentiating hippocampal neuronal precursor cells. They made the striking observation that Disc1 suppression in neuronal precursors resulted in overmigration, aberrant integration, and misfiring. But by what means? The papers by Kim et al. (2009) and Enomoto et al. (2009) in this issue of Neuron demonstrate that the critical function of DISC1 in postnatal hippocampal neurogenesis is mediated in large part by interaction with KIAA1212, known also and more evocatively as Girdin (Girders of actin filaments). Kim et al. (2009) further demonstrate that this interaction suppresses AKT signaling and, remarkably, that the gross effects of Disc1 suppression can be largely rescued by rapamycin, which inhibits mTOR, an effector pathway activated by AKT signaling.

Girdin Gets In on the AKT

Enomoto and colleagues had previously reported that AKT1, a key serine/threonine-specific kinase with multiple signaling properties, regulates actin organization and cell motility via Girdin, which directly binds actin at the leading edge of migrating cells. Drawing upon the evidence from a yeast two-hybrid screen that Girdin/KIAA1212 is a putative DISC1 interactor (Camargo et al., 2007), both Enomoto et al. (2009) and Kim et al. (2009) demonstrate physiological interaction between DISC1 and Girdin, albeit with some disagreement over the DISC1 interaction domains. Enomoto et al. (2009) show that in postnatal rodent brain Girdin is predominantly expressed in the dentate gyrus and pyramidal cell layers CA1 and CA3 of the hippocampus, mirroring DISC1 expression. Girdin affects angiogenesis and knockout mice do not survive into adulthood, but examination of early postnatal brain demonstrated a profound effect on development of the dentate gyrus. Uniquely in the brain, the dentate gyrus is formed postnatally and neurogenesis is continual. Examination of Girdin-deficient mice and experimental suppression of Girdin by retroviral-mediated siRNA demonstrated that Girdin regulates the migration and positioning of newborn dentate gyrus cells (Enomoto et al., 2009). Thus, in many, but not quite all respects, Girdin suppression mimics the effects of Disc1 suppression in adult neuronal progenitor cells, as reported previously by Duan et al. (2007). Enomoto et al. (2009) also reported that Disc1 suppression was associated with reduced Girdin immunoreactive protein at the growth cone, suggesting that DISC1 normally stabilizes and anchors Girdin there.
Counterintuitively, Kim et al. (2009) report a similar phenotype to Disc1 suppression upon overexpression of Girdin, but this perhaps indicates that both the balance of DISC1 interactome expression and the resultant stoichiometry of interactors, as well as the precise developmental timing of expression, is important.

A last point of apparent disagreement between the two studies relates to the role of AKT signaling. Enomoto et al. (2009) show that expression of a dominantly acting form of AKT does, as expected, abolish neuronal polarity, but this could not rescue the axonal defect in Girdin-suppressed neurons. Consistent with this, they report that the level of phosphorylated, active AKT in Girdin knockout mice is unaltered, arguing that Girdin acts downstream of AKT. By contrast, Kim et al. (2009) present multiple strands of evidence that DISC1 interaction with Girdin does indeed regulate AKT activity, as measured by phosphorylated AKT at Ser473, and by phosphorylation of S6, a well-characterized downstream target of mammalian target of rapamycin (mTOR)-AKT. Using the retroviral infection strategy to target proliferating progenitor cells in the dentate gyrus, they also showed that phenotypes of Disc1 suppression, namely increased soma size and number of primary dendrites, were mimicked by three strategies designed to activate AKT signaling: (1) expression of constitutively active AKT, (2) suppression of PTEN, a known suppressor of AKT activation, and (3) overexpression of Girdin. Moreover, they show that Girdin activity is modulated by DISC1 and, remarkably, that the effect of Disc1 suppression in dentate gyrus cells could be rescued by rapamycin. Rapamycin is the eponymous inhibitor of the mTOR function of AKT. Rapamycin is a potent immunosuppressant, so it is unlikely to be appropriate for chronic human use, but interestingly both the synaptic plasticity deficit and the cognitive behavior deficit in a mouse model of tuberous sclerosis were reportedly rescued after brief treatment with rapamycin (Ehninger et al., 2008).

**AKT, GSK3β, and Schizophrenia**

The genetic evidence for AKT being a risk factor for schizophrenia is modest by comparison to DISC1, but there is a growing body of evidence from human and mouse studies that the AKT pathway may indeed be important (reviewed by Arguello and Gogos, 2008). Genetic variants in AKT1 have been reported to be associated with schizophrenia. AKT1 activity and AKT-dependent phosphorylation of GSK3β is decreased in postmortem schizophrenic brains. Akt1 knockout mice show impaired prepulse inhibition of the startle response, a core-olary of the altered salience typifying schizophrenia, which is exacerbated by amphetamine (which can induce psychosis on chronic exposure in humans) and responds poorly to DRD2 (but not DRD1) agonists, the target of most current antipsychotic drugs. By contrast, both typical and atypical antipsychotics enhance AKT signaling by activating AKT or by increasing phosphorylation of GSK3β.

Mao et al. (2009) recently identified GSK3β as a novel DISC1 interactor, bringing the wnt pathway and β-catenin neurosignaling firmly to the fore. GSK3β is inhibited by both AKT signaling and by DISC1. It is a known target for the widely used mood stabilizer Lithium Chloride. Mao et al. (2009) further reported that administration of the GSK3β-specific inhibitor SB216763 could rescue the behavioral effects of lentivirally induced Disc1 suppression in the adult dentate gyrus. Interestingly, Kim et al. (2009) failed to see a rescuing effect of SB216763 on Disc1-suppressed newborn dentate granule cells. This suggests that DISC1 (and indeed different DISC1 interactors) may have different premitotic and postmitotic effects on adult neurogenesis. When all the data are taken together, we now have a tantalizing picture of how DISC1, through AKT, GSK3β, and other protein partners yet to be fully described, may regulate both neurodevelopment and neurotransmission, two core yet often opposed concepts in schizophrenia etiology. If the rather remarkable effects of rapamycin reported by Kim et al. (2009) and of SB216763 reported by Mao et al. (2009) are positive portents of future therapeutic strategies, it will nevertheless be critical to determine exactly which aspects of the DISC1 pathway phenotype must be corrected, and when, during brain development.

**What Else and What Next?**

The structure of DISC1 is not yet solved, but we know that DISC1 comprises a highly disordered N-terminal head domain and a C-terminal tail with multiple coiled-coil domains (reviewed in Chubb et al., 2008). This protein is built for protein-protein interaction. Millar et al. (2005) previously reported a genetic and biochemical link between DISC1 and PDE4B in modulating cAMP signaling through multiple binding sites specific to different isoforms of PDE4 (Murdoch et al., 2007). Mice display distinct behaviors and pharmacological responses of a schizophrenic-like or mood disorder nature depending upon which PDE4 binding domain is mutated (Clapcote et al., 2007). Intriguingly, the binding domains for PDE4, GSK3β, and now Girdin at least partially overlap. Both DISC1 and Girdin dimerize and both bind NDEL1, which in turn binds NDE1 and thus LIS1, yet another key protein in brain development. DISC1 forms higher-order multimers, a process that appears to alter the binding of NDEL1 and is sensitive to polymorphic variation at the Ser704Cys position (Leiiveld et al., 2009). This polymorphism has been previously related by fMRI and working memory tasks to differential hippocampal engagement in normal human subjects, and also to altered brain expression of DISC1 partners (reviewed in Chubb et al., 2008). Variants of DISC1, PDE4B, PDE4D, and NDE1 are genetic risk factors in their own right (reviewed in Chubb et al., 2008) and are also transcriptional modulators of cytoskeletal, synaptogenic, neurodevelopmental, and sensory perception proteins (Hennah and Porteous, 2009). This set of proteins is significantly enriched for current targets of psychiatric drug development (Hennah and Porteous, 2009). When considering what DISC1 interacts with, and where and when it does so, it is important also to take account of the growing evidence for multiple transcripts and protein isoforms of DISC1 and their developmental regulation (reviewed in Chubb et al., 2008). This will have a direct bearing upon the capacity for DISC1 to self-assemble and to bind potential interactors. Self-evidently, considering DISC1 interactions only in pairwise fashion is bound to oversimplify and potentially deceive. Defining the DISC1 proteome by cell type.
and lineage during prenatal and postnatal development and in the adult brain may be necessary for a full understanding of the multiplicity of DISC1 functions, how these relate to psychopathology, and what that means for treatment strategies.

The DISC1 Proteome: A Suite in G Minor?
The DISC1 pathway is relevant to many of the saddest and most tragic human conditions—schizophrenia, bipolar disorder, and major depression at least, and possibly autism too. The emerging picture is of DISC1 as the orchestrator of a suite of protein-protein interactions harmonized in time and space. Extending the musical analogy to schizophrenia, if the conductor is off tempo, no matter how able the symphony of brain players, the net result will be off key. And even if the DISC1 conductor is on tempo, if any of the key players are out of tune, then the brain performance may be perceived as similarly discordant.

It now becomes critical to establish the biophysical state of the DISC1 complex in both time and place, the valency of protein-interaction, and the extent to which this is independent, cooperative, or competitive. We speculate that the mechanism by which the DISC1 interactome regulates pathways of action is to sequester and compartmentalize signaling pathways on a cell-by-cell and cell compartment-by-cell compartment basis. In this model, we are replacing the conventional depiction of linear pathways, feedback loops, and “upstream/downstream” (see for example Figure 7 in Kim et al., 2009) with the concept that the DISC1 complex is the pathway, whether in the nucleus, at the growth cone, the centrosome, the mitochondria, or the presynaptic or postsynaptic density. Each pathway or suite is defined by local and locally determined isoforms, interactions, cAMP concentrations, and phosphorylation states. To test and refine this will require an even finer level of cellular molecular anatomy, but it is around this concept that we would seek and expect to devise molecular therapies that were both effective and safe. Ongoing structure-function analyses will be aided by current, major efforts now underway to define causal clinical variants in the DISC1 pathway through genomic resequencing. Major challenges remain, but remarkable progress has been made and further progress toward understanding and intervention will surely follow.

REFERENCES