DISC1 Partners with GSK3β in Neurogenesis

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The protein DISC1, encoded by a gene implicated in schizophrenia susceptibility, regulates the development of postmitotic neurons. Mao et al. (2009) now report that DISC1 also regulates the proliferation of embryonic and adult neural progenitor cells through the GSK3β/β-catenin pathway, providing new insights into how susceptibility genes may contribute to the etiology of psychiatric disorders.

Schizophrenia is a severe, chronic, and disabling mental illness with a prominent genetic basis (Ross et al., 2006). Over the past decade, the hypothesis that schizophrenia is a disease of neuronal development has gained momentum (Marenco and Weinberger, 2000). A key susceptibility gene for schizophrenia is *DISC1* (*Disrupted in Schizophrenia 1*), originally identified at the (1;11)(q42;q14) translocation breakpoint that cosegregates with schizophrenia and other major affective disorders in a large Scottish family (Chubb et al., 2008). Genetic linkage studies have implicated mutations in *DISC1* as a general risk factor for schizophrenia, schizoaffective disorder, bipolar disorder, major depression, and autism spectrum disorders. But how dysfunction of the DISC1 protein could contribute to this broad spectrum of psychiatric disorders remains unclear. In vivo studies have shown that DISC1 regulates multiple steps in neurogenesis, including the morphogenesis, maturation, migration, and synaptic integration of neurons (Duan et al., 2007; Faulkner et al., 2008; Kamiya et al., 2005). In this issue of *Cell*, Mao et al. (2009) provide compelling evidence that DISC1 also regulates the initial step of neurogenesis, that is, the proliferation of neural progenitor cells during brain development in the mouse embryo. Moreover, loss of function of DISC1 in the dentate gyrus (part of the hippocampus) of adult mice results in reduced neural progenitor cell proliferation and the appearance of schizophrenic and depressive-like behaviors.

DISC1 was first implicated in neuronal development through its expression pattern in the developing mammalian brain and through the biochemical identification of interacting proteins that are involved in centrosome assembly, cytoskeletal reorganization, and synaptic functions (reviewed in Chubb et al., 2008). In vitro and in vivo studies examining the functions of DISC1 have largely focused on postmitotic neurons. In their new work, Mao et al. (2009) noticed that DISC1 is also highly expressed in neural progenitor cells residing in the ventricular and subventricular zones of mouse embryonic brain. Starting with cultured neural progenitor cell lines derived from adult rat hippocampal tissue, these investigators found that expression in these cells of short hairpin RNAs (shRNAs) directed against DISC1 dramatically decreased their proliferation, whereas overexpression of human DISC1 promoted proliferation. Continuing in vivo, the authors introduced the shRNA constructs into mouse brains at embryonic day 13 (E13) of development and discovered a substantial reduction in the mitotic index of cells within the ventricular and subventricular zones. Importantly, they could rescue such defects with human DISC1, thus ensuring the specificity of DISC1 knockdown by the shRNAs.

Detailed analysis showed that decreased neural progenitor cell proliferation is due to accelerated exit from the cell cycle and premature differentiation into neurons, suggesting that DISC1 controls the tempo of neurogenesis during embryonic cortical development. Mao and colleagues also found a reduction in bromodeoxyuridine incorporation after lentivirus-mediated expression of DISC1 shRNA in the adult mouse dentate gyrus, indicating reduced proliferation of adult neural progenitor cells. Given that lentiviruses can infect many cell types in the dentate gyrus of adult mammals, it remains to be determined whether DISC1 affects the proliferation of specific cell types, such as quiescent neural stem cells, transient amplifying cells, or neuroblasts, and whether DISC1 suppression in mature dentate granule cells contributes to the observed defects in neurogenesis. Consistent with earlier work (Duan et al., 2007), Mao et al. also observe aberrant positioning and increased complexity of the dendritic morphology of dentate granule cells that lack DISC1.

How does DISC1 regulate the proliferation of neural progenitor cells? One clue comes from early findings that the Wnt/β-catenin signaling pathway regulates the maintenance and differentiation of neural progenitors in the central nervous system (Chenn and Walsh, 2002). Indeed, Mao et al. now find that DISC1 regulates β-catenin abundance and is required for Wnt3a-induced proliferation and activation of downstream transcription factors of the LEF/TCF family in cultured adult neural progenitor cells (Figure 1). Importantly, the phenotype of neural progenitor cells lacking DISC1 can be rescued by overexpression of degradation-resistant β-catenin in vivo. The final piece of the puzzle arrived with the identification of glycogen synthase

kinase 3β (GSK3β) as a direct binding partner for DISC1 (Figure 1). In elegant experiments in vitro, Mao et al. provide compelling evidence that DISC1 blocks GSK3β activity, and they pinpoint a 15-mer domain within the N terminus of DISC1 that interacts with GSK3β. They then demonstrated the physiological significance of this interaction in vivo: a chemical inhibitor of GSK3β rescued the defect in neural progenitor cell proliferation induced by DISC1 suppression in both mouse embryonic cortex and adult dentate gyrus.

The Mao et al. study suggests a model in which DISC1 regulates neural progenitor cell proliferation by interacting with and inhibiting GSK3β, which in turn results in stabilization of β-catenin and activation of downstream transcription factors that prevent premature exit from the cell cycle and neuronal differentiation (Figure 1). This study is important because it identifies GSK3β as a target of DISC1 and puts DISC1 at the center of a network of different signaling pathways. This work also broadens our understanding of DISC1 functions in neural development and further implicates DISC1 as a key player in the etiology of mental illness. Finally, the new study unifies the role of DISC1 as a temporal regulator of neurogenesis, controlling processes from neural progenitor cell proliferation to cell cycle exit, neuronal differentiation, maturation, and integration of postmitotic neurons into the neural circuitry. The Mao et al. study has important implications for understanding how alterations in DISC1 signaling may contribute to the etiology of psychiatric disorders and offers potential avenues for therapeutic intervention. Converging evidence implicates misregulation of GSK3β in schizophrenia and related mental disorders (Beaulieu et al., 2008), and antipsychotic drugs and the mood stabilizer lithium (used to treat bipolar disorder) are known to alter GSK3β activity.

The new work by Mao et al. raises many questions, such as which signaling components act upstream of GSK3β. These investigators do provide in vitro evidence for the potential involvement of Wnt3a. Does DISC1-dependent proliferation of neural progenitor cells involve Wnts in vivo, and are additional pathways upstream of GSK3β such as the receptor tyrosine kinase-PI3K-AKT pathway involved (Figure 1)? Many signaling components in the latter pathway have been implicated in schizophrenia, including neuregulin 1, its receptor ErbB4, and AKT1 (reviewed in Ross et al., 2006). AKT phosphorylates GSK3β at serine residue 9 (Ser9), resulting in GSK3β inactivation. In proliferating neural progenitor cells, Ser9 phosphorylation is not affected by DISC1 knockdown, suggesting a minimal role for AKT during the proliferation phase. Whether this pathway is involved in DISC1-dependent regulation of neuronal development awaits future studies.

Probably the most surprising result of the Mao et al. study is the behavioral outcome after lentivirus-mediated DISC1 suppression in the mouse adult dentate gyrus. Cumulative studies have implicated adult hippocampal neurogenesis in learning, memory, and mood regulation (reviewed in Zhao et al., 2008). However, ablation of adult neurogenesis does not appear to have a causal effect on depressive behavior. Certain schizophrenia-related and depression-related phenotypes have been observed in rodents in which DISC1 expression has been manipulated during embryonic development (reviewed in Chubb et al., 2008). In contrast to previous studies, Mao et al. used lentiviruses to knockdown DISC1 expression specifically in the adult mouse dentate gyrus. These mice showed reduced cell proliferation in the dentate gyrus and exhibited hyperlocomotion in response to novel stimuli (a schizophrenia-like behavior) but greater immobility in the forced swim

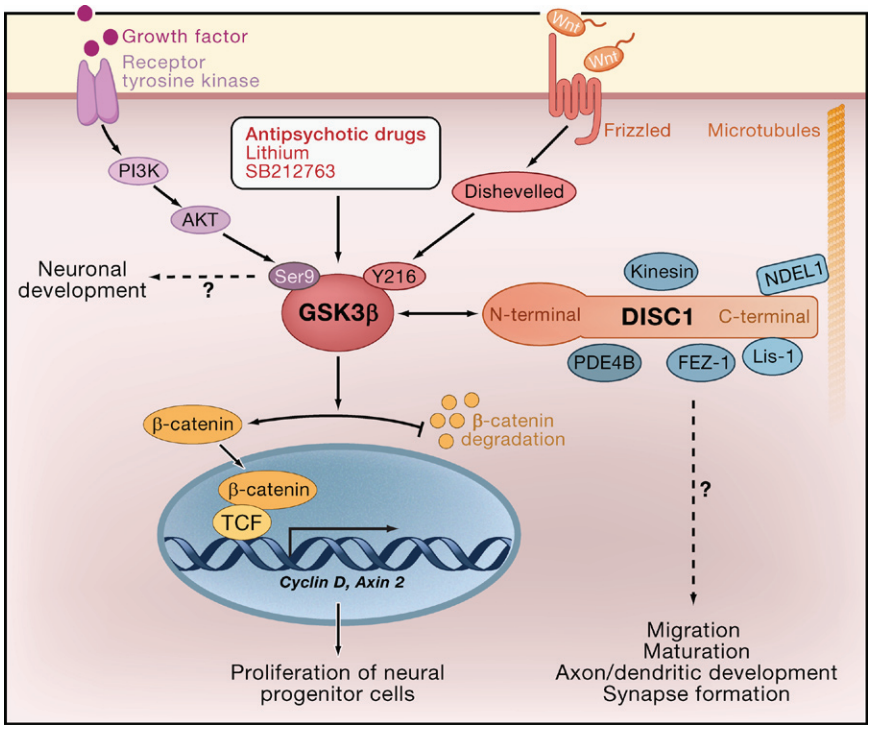

Figure 1. Regulation of Neurogenesis by DISC1

The model shows how DISC1 may regulate different steps in neurogenesis in embryonic and adult mouse brain. DISC1 inhibits GSK3β through its N-terminal domain, which results in stabilization of β-catenin and activation of downstream transcription factors. These factors promote proliferation of neural progenitor cells, preventing their premature exit from the cell cycle and neuronal differentiation. A candidate pathway working upstream of GSK3β is the Wnt signaling pathway, which regulates autophosphorylation of GSK3β at tyrosine 216 (Y216). GSK3β activity is also regulated by phosphorylation at serine residue 9 (Ser9) by the receptor tyrosine kinase (RTK)-PI3K-AKT pathway. Antipsychotic drugs, the mood stabilizer lithium, and drugs inducing psychosis alter GSK3β activity indirectly by different mechanisms. DISC1 also interacts with other proteins, including NDEL1, Lis-1, PDE4B, FEZ1, and kinesin. (PI3K, phosphoinositide-3 kinase; GSK3β, glycogen synthase kinase 3β; NDEL1, NudE-like 1; Lis-1, Lissencephaly-1; PDE4B, phosphodiesterase 4B; FEZ-1, fasciculation and elongation protein zeta 1.)

test (depression-like behavior). Importantly, both cellular and behavioral phenotypes could be normalized by treating the mice with a GSK3β inhibitor. These findings support an emerging view that dysfunction of adult hippocampal neurogenesis may contribute to manifestations of psychiatric disease during adulthood (Duan et al., 2007). An intriguing hypothesis is that defects in embryonic neuronal development predispose individuals to neuropsychiatric illness, whereas dysfunctional neurogenesis in the adult (which is dynamically regulated by the existing neuronal circuitry) pushes the individual over the threshold, resulting in the full manifestation of neuropsychiatric disease. Future genetic studies using conditional knockout mice to manipulate specific cell types in the adult brain will help to clarify this model. With many exciting new studies underway, the next chapter in the saga of DISC1 and its role in neural development should soon be here.

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Polycomb Repression under the Skin

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Polycomb group proteins control a hierarchy of gene expression during the differentiation of stem cells. In this issue, Ezhkova et al. (2009) show that repression mediated by Polycomb proteins controls the timing of differentiation of precursor cells of the epidermal lineage.

Pluripotent cells in the early embryo have the capacity to differentiate into any cell type to produce all the tissues of the adult body but are prevented from doing so until the appropriate time and place. Initially, the suppression of all the various potential pathways of differentiation is transient, but once a single pathway of differentiation has been selected, this suppression is made permanent. In embryonic stem (ES) cells nearly all genes involved in differentiation are bound by Polycomb-repressive complexes 1 and 2 (PRC1 and PRC2) and are marked by histone H3 lysine 27 trimethylation (H3K27me3) (Boyer et al., 2006). This discovery suggests that mechanisms involving Polycomb group (PcG) proteins prevent indiscriminate differentiation of ES cells (Figure 1). But experiments in which PcG gene function is knocked down show that the picture is more complicated at later stages of cell commitment. In this issue of *Cell*, Ezhkova et al. (2009) show that the loss of PcG function in epidermal precursor cells specifically derepresses genes relevant to epidermal differentiation, thereby accelerating the rate of normal maturation.

In ES cells, genes involved in differentiation that are bound by PcG complexes not only bear the repressive H3K27me3 mark but also are trimethylated at lysine 4 of histone H3 (H3K4me3), a mark associated with transcriptional activity. The simultaneous presence of both repressive and active chromatin marks, called the bivalent state (Bernstein et al., 2006), is in fact accompanied by at least some transcriptional activity, suggesting that it signifies an uneasy equilibrium between opposing forces. Indeed, multiple reports have shown that when PcG function is knocked down in ES cells, the expression of genes controlling many differentiation pathways is partially derepressed. Embryos deficient in Ezh2, the histone methyltransferase component of PRC2, fail to progress beyond implantation and do not allow the establishment of ES cells (O'Carroll et al., 2001).

Is PcG repression then essential to maintain the pluripotent state? Surprisingly, it is possible to generate relatively stable ES cells lacking Suz12 or Eed, which are essential components of the PRC2 complex, perhaps suggesting that Ezh2 has additional functions beyond the PRC2 complex. Although these cells are depleted of H3K27me3 and express higher levels of differentia-