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9.914 Special Topics: Genetics, Neurobiology, and Pathophysiology of Psychiatric Disorders
Fall 2008

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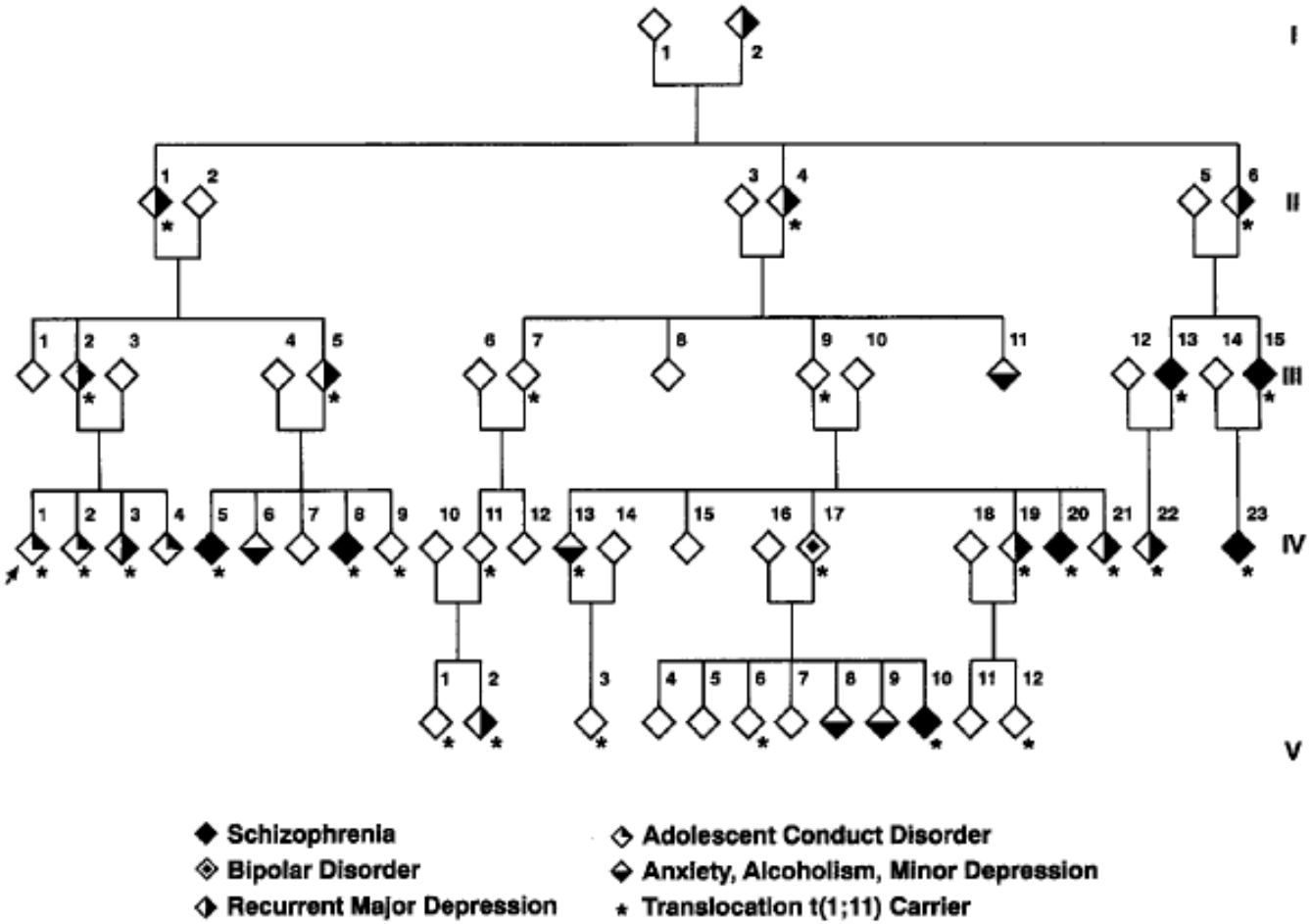
Disrupted in schizophrenia 1 (DISC1)



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- **Chromosomal translocation (1;11)(q42;q14.3) in a large Scottish family affected by schizophrenia, major depression and bipolar disorder (Blackwood et al., *Am. J. Hum. Genet. 69:428–433, 2001)***

Disrupted in schizophrenia 1 (DISC1)

•18 of 29 translocation carriers are diagnosed with major mental Illness (schizophrenia (7), bipolar disorder (1), major recurrent depression (10)) whereas none of non-translocation carriers have such a diagnosis– t(1;11) a simple dominant mode of inheritance with reduced penetrance.

•In unaffected translocation carriers, the latency and amplitude of the event related potential (ERP) P300, is indistinguishable from that of affected individuals with the characteristic abnormal P300 ERP associated with schizophrenia and bipolar disorder—altered P300 a correlated endophenotype

Translocation break point is located in intron 8 of the DISC1 gene that disrupts the expression of DISC1 and DISC2

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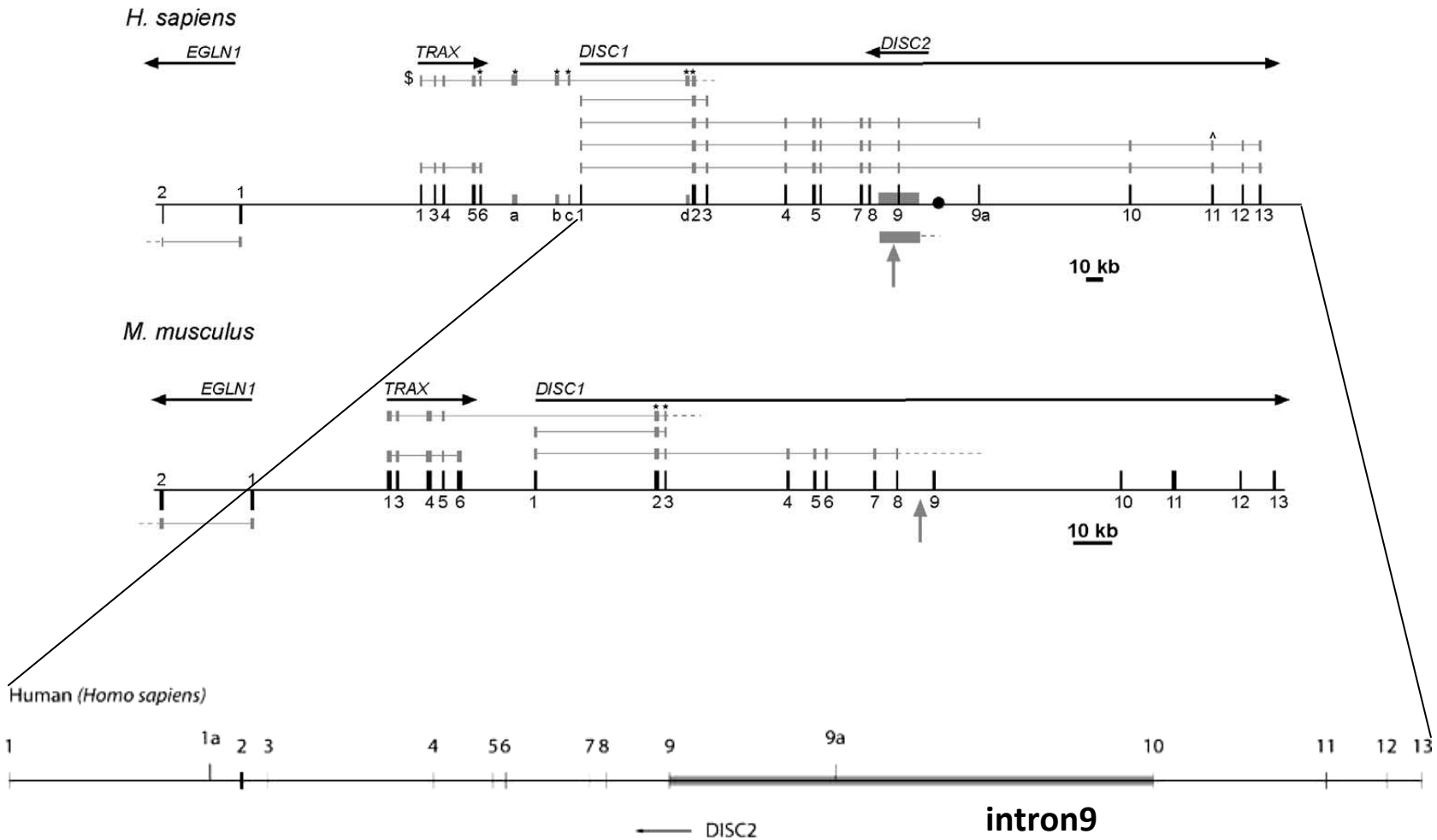
DISC2 encodes a non-coding RNA which is also disrupted by the translocation (only in humans, not conserved in mouse)



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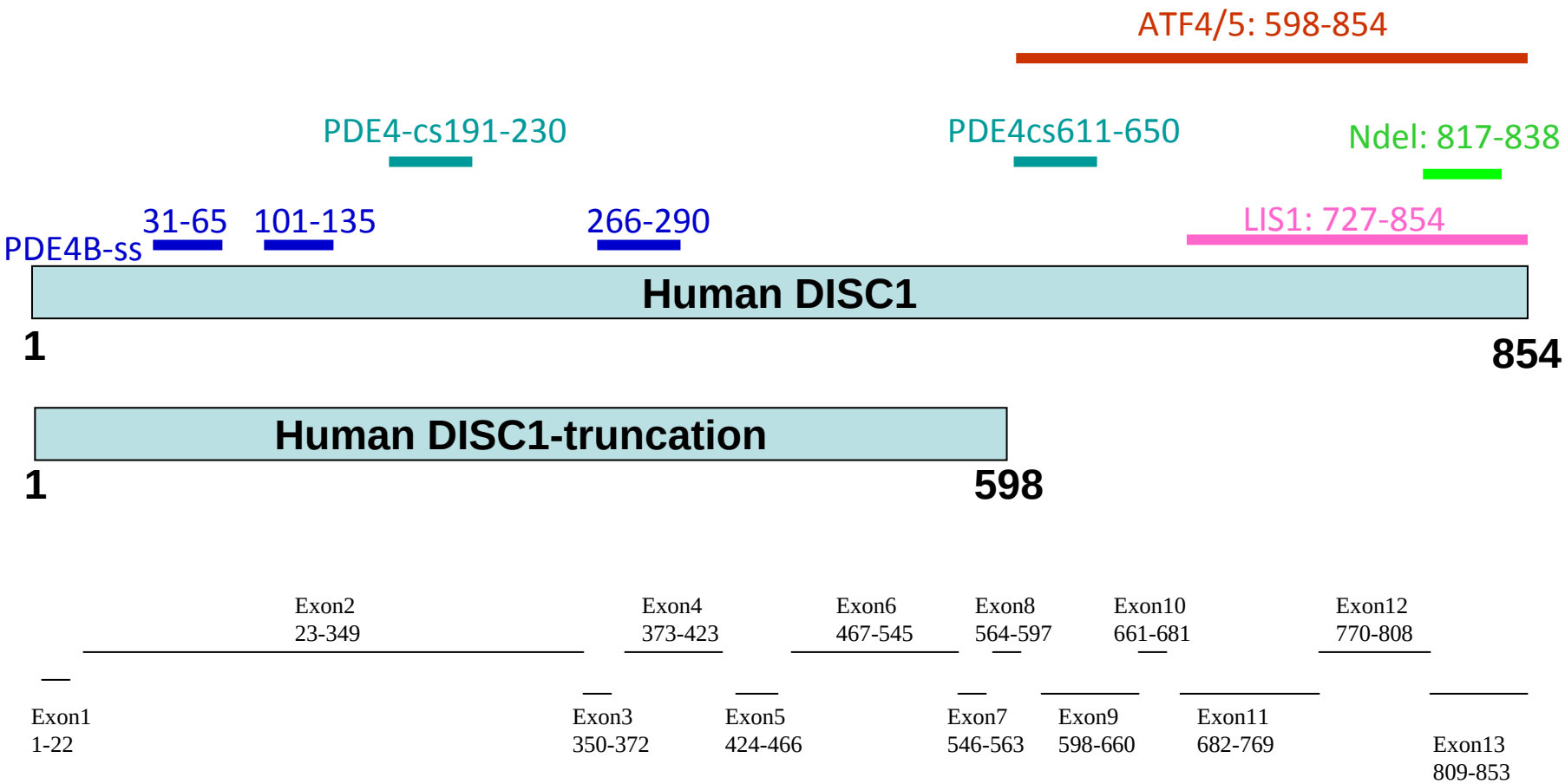
Protein structure of human DISC1



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Dominant negative or haploinsufficiency?

- In the lymphoblastoid cell lines derived from translocation carriers, both the DISC1 transcript and protein levels are reduced
- DISC1 antibody failed to detect the presence of the truncated DISC1 protein in these lymphoblastoid cells lines
- Needs to be confirmed using brain tissues

Genetic linkage and association of DISC1 with mental illnesses

- Association with Finnish schizophrenia families (221 families, 557 affected individuals) (*Hum Mol Genet. 2001. 10:1611*); Japanese (*Neurosci Lett*. 2004;368:41), North American white population (*Am J Hum Genet*. 2004;75:862) and Chinese (*J Psychiatr Res*. 2006). Several reports also indicate association of DISC1 with bipolar disorder, major depression and autism spectrum disorder.

- However, all of these studies suffer from small sample size with insufficient power to reach significance.

- So far, P Sklar fails to observe meaningful genetic linkage or association of DISC1 with schizophrenia or BP

Identification of high risk DISC1 structural variants with a 2% attributable risk for schizophrenia

Wenjia Song [a], Wenyan Li [a], Jinong Feng [a], Leonard L. Heston [b], William A. Scaringe [a], Steve S. Sommer [a,*]

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Table 1
Cohort-specific variants found in Caucasian schizophrenia patients and controls

#	NT change	AA change	Exon/intron	Conservation[b]	Patient ID No.	Gender	Cases	Controls	Screened control alleles
S#1	41 G > C	G14A	Exonl	Ma.	S434	M	1/288	0/288	0/10,000
S#2	109 C > T	R37W	Exon2	Ma.D.Mo.	S113	F	1/288	0/288	0/10,000
S#3	269 C > T	S90L	Exon2	D.	S220 S432	M M	2/288	0/288	0/10,000
					S432	M			
S#4	1253 G > A	R418H	Exon4	Ma.F.Z	S044#2	M	1/288	0/288	0/10,000
S#5	1808 C > T	T603I	Exon9	Ma.D.Mo.	S155	M	1/288	0/288	0/10,000
S#6	248 C > T	A83V	Exon2	D.Mo.	S508	M	1/288	0/288	5/2000[c]
S#7	1295 C > T	P432L	Exon5	Ma.	S011	M	1/288	0/288	$\geqslant$15/6400[a,c]
C#1	790 C > T	Q264C	Exon2	Ma.	C110		0/288	1/288	5/6400[c]

DISC1 in neuronal migration and dendrite development– RNAi knockdown and overexpression of the truncation mutant

nature cell biology ARTICLES

A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development

Atsushi Kamiya[1,2], Ken-ichiro Kubo[3], Toshifumi Tomoda[4,5], Manabu Takaki[1], Richard Youn[1], Yuji Ozeki[1,2], Naoya Sawamura[1], Una Park[6], Chikako Kudo[3,7], Masako Okawa[2], Christopher A. Ross[1,6,8,9], Mary E. Hatten[4], Kazunori Nakajima[3,7] and Akira Sawa[1,6,9,10]

Disrupted-In-Schizophrenia-1 (DISC1), originally identified at the breakpoint of a chromosomal translocation that is linked to a rare familial schizophrenia, has been genetically implicated in schizophrenia in other populations. Schizophrenia involves subtle cytoarchitectural abnormalities that arise during neurodevelopment, but the underlying molecular mechanisms are unclear. Here, we demonstrate that DISC1 is a component of the microtubule-associated dynein motor complex and is essential for maintaining the complex at the centrosome, hence contributing to normal microtubular dynamics. Carboxy-terminal-truncated mutant DISC1 (mutDISC1), which results from a chromosomal translocation, functions in a dominant-negative manner by redistributing wild-type DISC1 through self-association and by dissociating the DISC1–dynein complex from the centrosome. Consequently, either depletion of endogenous DISC1 or expression of mutDISC1 impairs neurite outgrowth *in vitro* and proper development of the cerebral cortex *in vivo*. These results indicate that DISC1 is involved in cerebral cortex development, and suggest that loss of DISC1 function may underlie neurodevelopmental dysfunction in schizophrenia.

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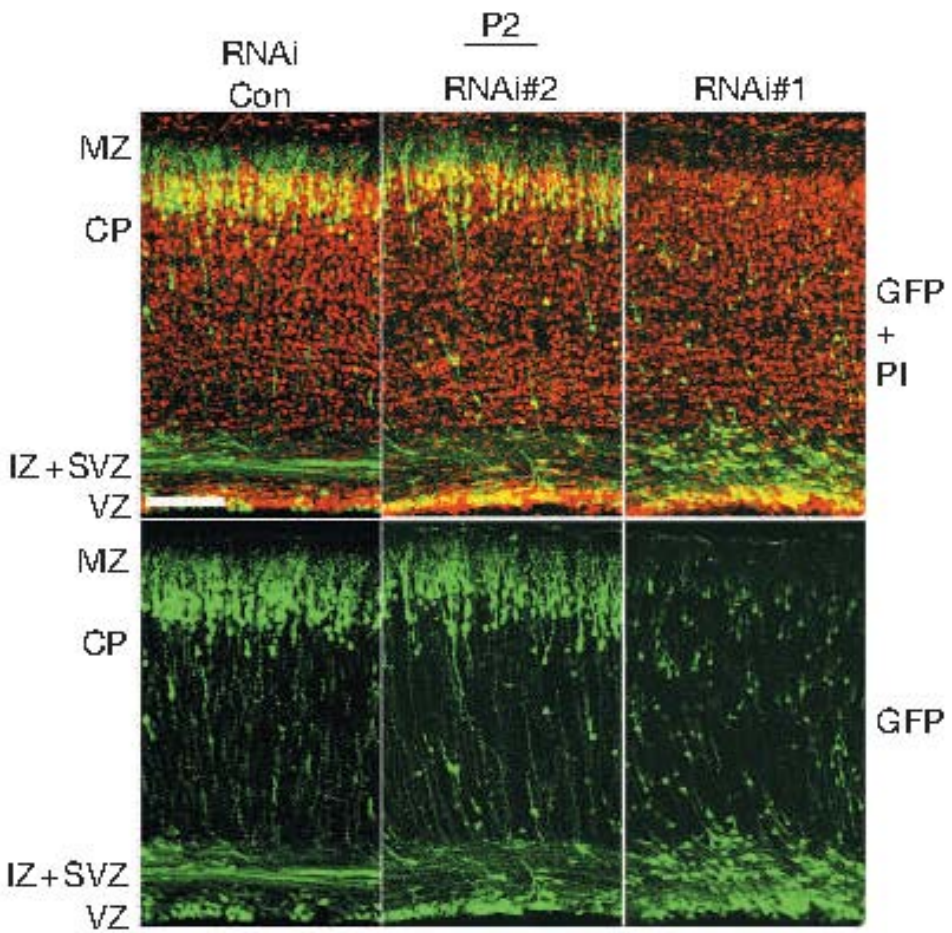
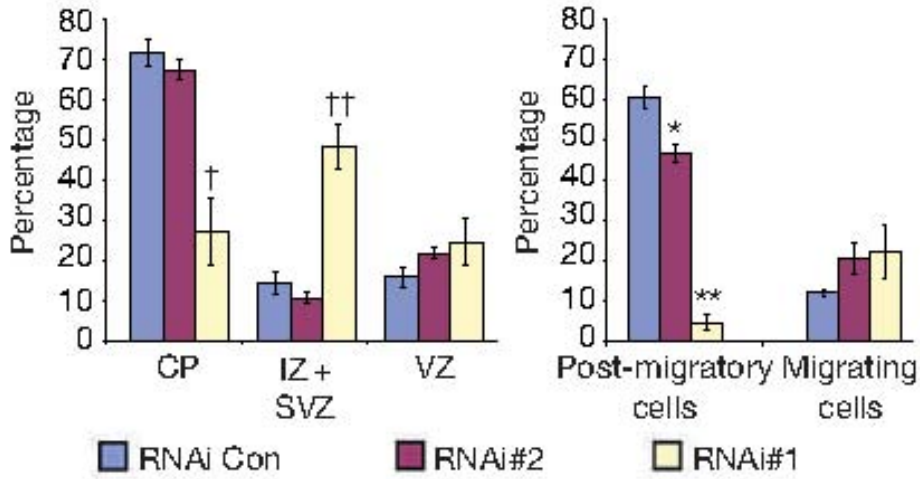
DISC1 in neuronal migration and dendrite development– RNAi knockdown and overexpression of the truncation mutant





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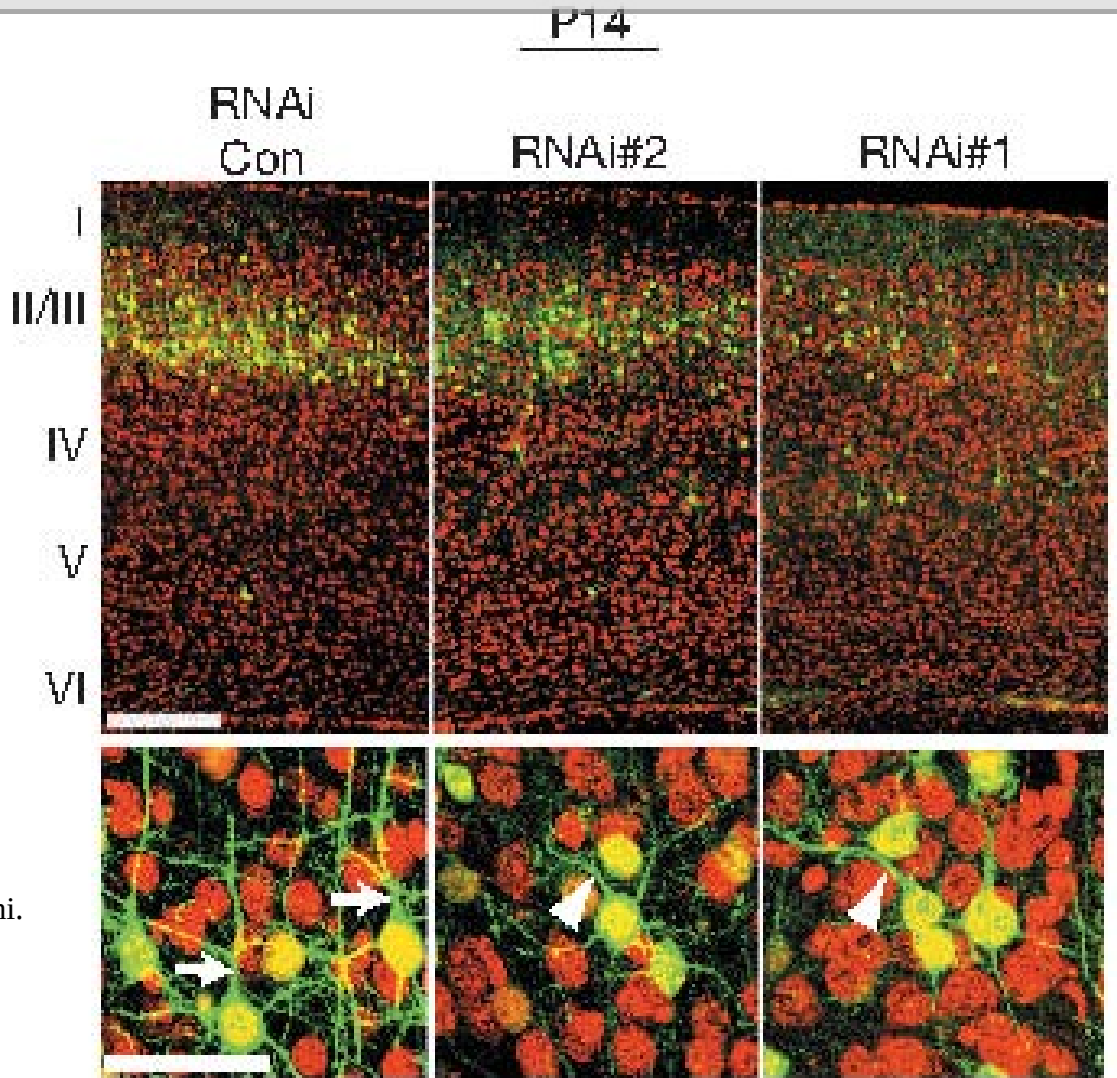
DISC1 in neuronal migration and dendrite development– RNAi knockdown and overexpression of the truncation mutant



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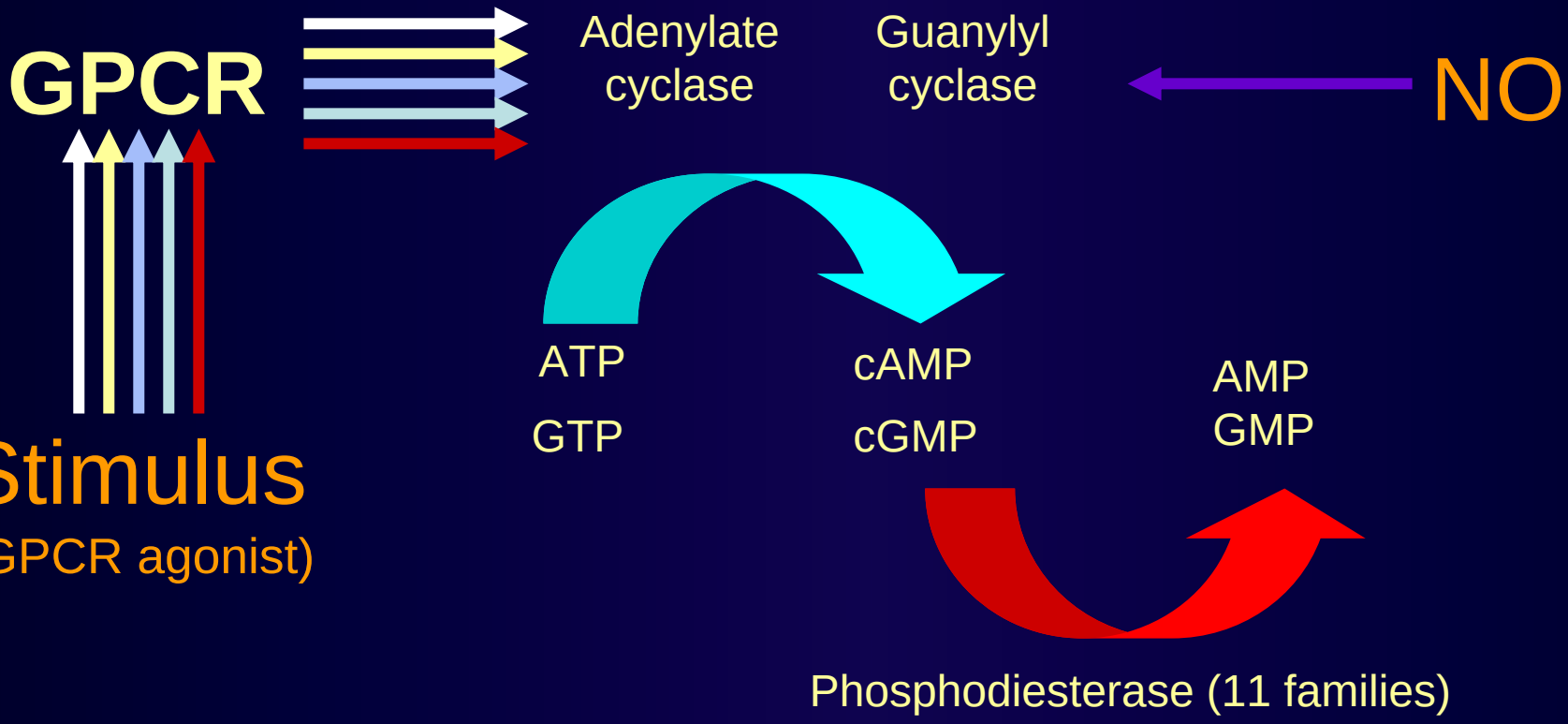
Phosphodiesterases (PDEs)

PDEs are the sole means of inactivating cAMP signaling



cAMP signaling compartmentalization

Regulate localization, duration, and amplitude of cyclic nucleotide signaling within subcellular domains

Phosphodiesterases (cont.)

Clinically relevant

PDE1 inhibitors
Vascular disease

PDE3 inhibitors
Heart failure

PDE5 inhibitor
Viagra

PDE4 inhibitors
Asthma
Pulmonary hypertension
COPD
Osteoporosis
Depression (in mice) → Rolipram has side effects in humans (vomiting and nausea)

Interaction of DISC1 and PDE4B is regulated by cAMP

Figure removed due to copyright reasons.

Interaction of DISC1 with ATF4

Nuclear DISC1 regulates CRE-mediated gene transcription and sleep homeostasis in the fruit fly

N Sawamura[1,2], T Ando[3], Y Maruyama[3], M Fujimuro[4], H Mochizuki[3], K Honjo[3], M Shimoda[5], H Toda[3,6], T Sawamura-Yamamoto[1], LA Makuch[7], A Hayashi[1], K Ishizuka[1], NG Cascella[1], A Kamiya[1], N Ishida[8], T Tomoda[6], T Hai[9], K Furukubo-Tokunaga[3] and A Sawa[1,7]

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Disrupted-in-schizophrenia-1 (DISC1) is one of major susceptibility factors for a wide range of mental illnesses, including schizophrenia, bipolar disorder, major depression and autism spectrum conditions. DISC1 is located in several subcellular domains, such as the centrosome and the nucleus, and interacts with various proteins, including NudE-like (NUDEL/NDEL1) and activating transcription factor 4 (ATF4)/CREB2. Nevertheless, a role for DISC1 *in vivo* remains to be elucidated. Therefore, we have generated a *Drosophila* model for examining normal functions of DISC1 in living organisms. DISC1 transgenic flies with preferential accumulation of exogenous human DISC1 in the nucleus display disturbance in sleep homeostasis, which has been reportedly associated with CREB signaling/CRE-mediated gene transcription. Thus, in mammalian cells, we characterized nuclear DISC1, and identified a subset of nuclear DISC1 that colocalizes with the promyelocytic leukemia (PML) bodies, a nuclear compartment for gene transcription. Furthermore, we identified three functional *cis*-elements that regulate the nuclear localization of DISC1. We also report that DISC1 interacts with ATF4/CREB2 and a corepressor N-CoR, modulating CRE-mediated gene transcription.

Molecular Psychiatry advance online publication, 2 September 2008; doi:10.1038/mp.2008.101

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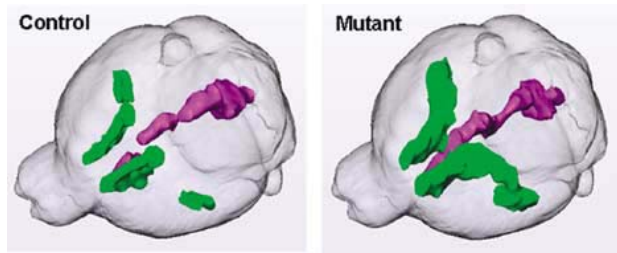
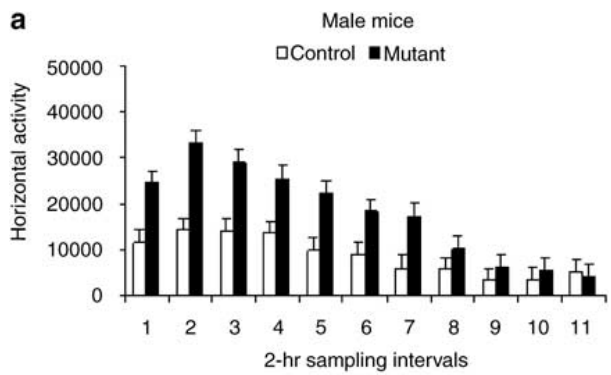
DISC1 mouse models

- Transgenic models:
  - A Sawa et al (PNAS, 2007, 104)
    - DN-DISC1 was driven by the CaMKII promoter
    - enlarged lateral ventricles
    - hyperactivity, disturbance in sensorimotor gating and olfactory-associated behavior, and an anhedonia/depression-like deficit
  - C Ross et al (Mol. Psychiatry, 2008,13)
    - Tet-off inducible DISC1 truncation driven by CaMKII promoter
    - a mild enlargement of the lateral ventricles and attenuation of neurite outgrowth
    - spontaneous hyperactivity and alterations in social interaction, deficient spatial memory





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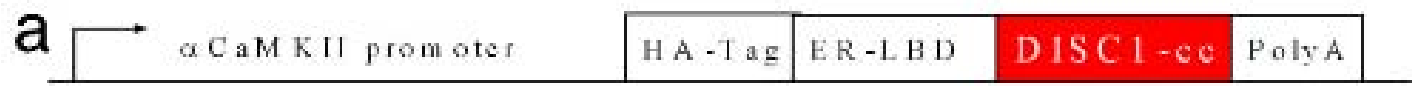
DISC1 mouse models-cont.

- Transgenic models:
  - Li et al (PNAS, 2007, 104, 18282)
    - C-terminal portion of DISC1 (aa 671-852) was fused to estrogen receptor and driven by the CaMKII promoter



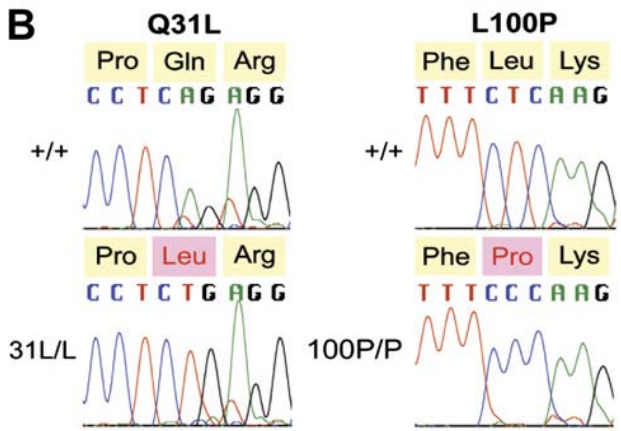
1 figure from Li, et al. "Specific Developmentaldisruption Of Disrupted-In-Schizophrenia-1 Function Results In Schizophrenia-Related Phenotypes In Mice." *Proc Natl Acad Sci* 104, no. 46 (2007): 18280-5. Copyright (copyright year) National Academy of Sciences, U.S.A."

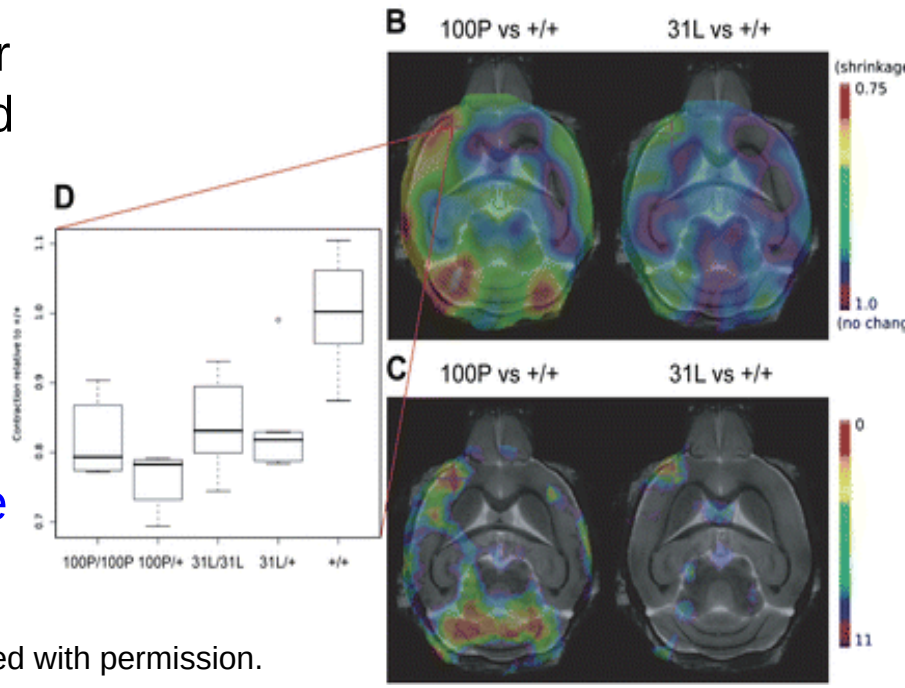
    - Induction of expression for only 24 hr at P7
    - reduced hippocampal dendritic complexity in adult
    - depressive-like traits, abnormal spatial working memory, and reduced sociability.
    - Results suggest that alterations of DISC1 function during brain development contribute to behavioral phenotypes

DISC1 mouse models-cont.

Clapcote et al (Neuron, 2007, 54, 387)

- Screen for DISC1 exon 2 mutants after ENU induced mutagenesis—found 2 different mutant strains harboring Q31L and L100P mutations, respectively
- Reduced cortical size and enlarged ventricles
- Q31L showed depressive-like behavior with deficits in the forced swim test and other measures
- L100P mutant mice exhibited schizophrenic-like behavior, with profound deficits in prepulse inhibition and latent inhibition
- However, neither of these residues are conserved in humans





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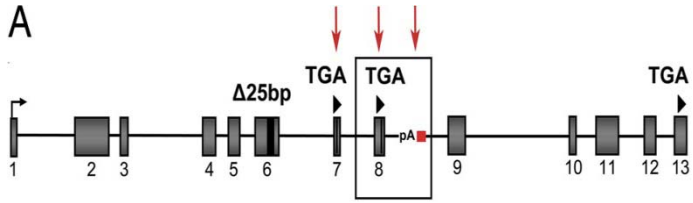
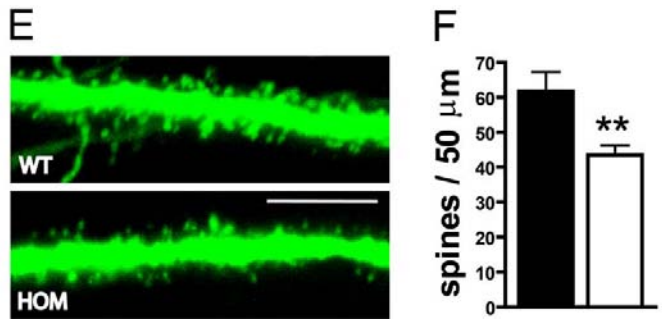
DISC1 mouse models-cont.

- Deletion model:
  - Kvajo et al (PNAS, 2008, 105, 7076)
    - 129/Sv strain has termination codon at exon 7 that precludes expression of full length gene product
    - two termination codons (in exons 7 and 8) and a premature polyadenylation site in intron 8, which leads to the production of a truncated transcript
    - alterations in the organization of newly born and mature neurons in the dentate gyrus.
    - Reduced dendritic spine density
    - Impairment in short term plasticity and working memory





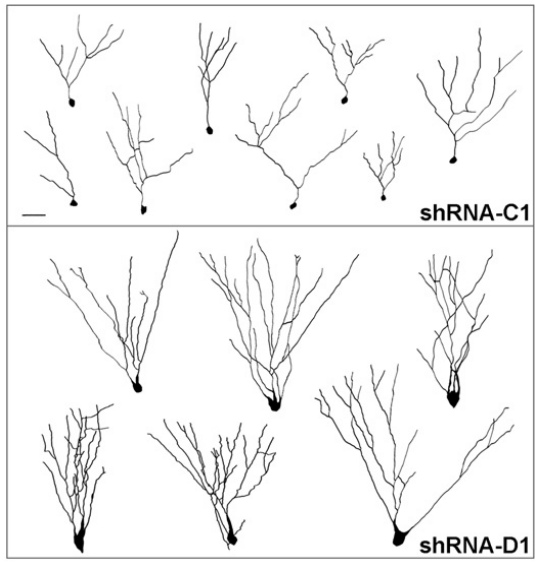
2 figures from Kvajo, M., et al. Proc Natl Acad Sci (2008)
Fig. 1A, Fig. 3E, Fig. 3F from Kvajo, M., et al. "A Mutation In Mouse Disc1 That Models A Schizophrenia Risk Allele Leads To Specific Alterations In Neuronal Architecture And Cognition" *Proc Natl Acad Sci* 105, no. 19 (2008): 7076-81. Copyright (copyright year) National Academy of Sciences, U.S.A."

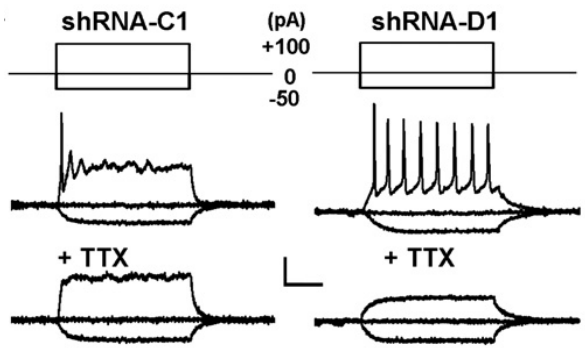
Caveat: many alternatively spliced DISC1 products are produced in this strain. Thus, this is a partial loss of function model

•DISC1 complete deletion model has not been possible to produce

DISC1 in integration of newly born neurons during adult neurogenesis

- **Duan et al (Cell, 2007, 130:1146)**
  - DISC1 RNAi knockdown leads to accelerated migration and mis-positioning of newly born neurons during adult neurogenesis in the dentate gyrus
  - newborn neurons with DISC1 knockdown exhibit enhanced excitability and accelerated dendritic development and synapse formation
  - Interactions with Nudel and Lis1 essential for the function of DISC1





•These results obtained from DISC1 loss of function in adult brain are opposite from those of A Sawa et al and A. Silva et al where they showed that DISC1 loss of function impaired neuronal migration and reduced dendritic complexity

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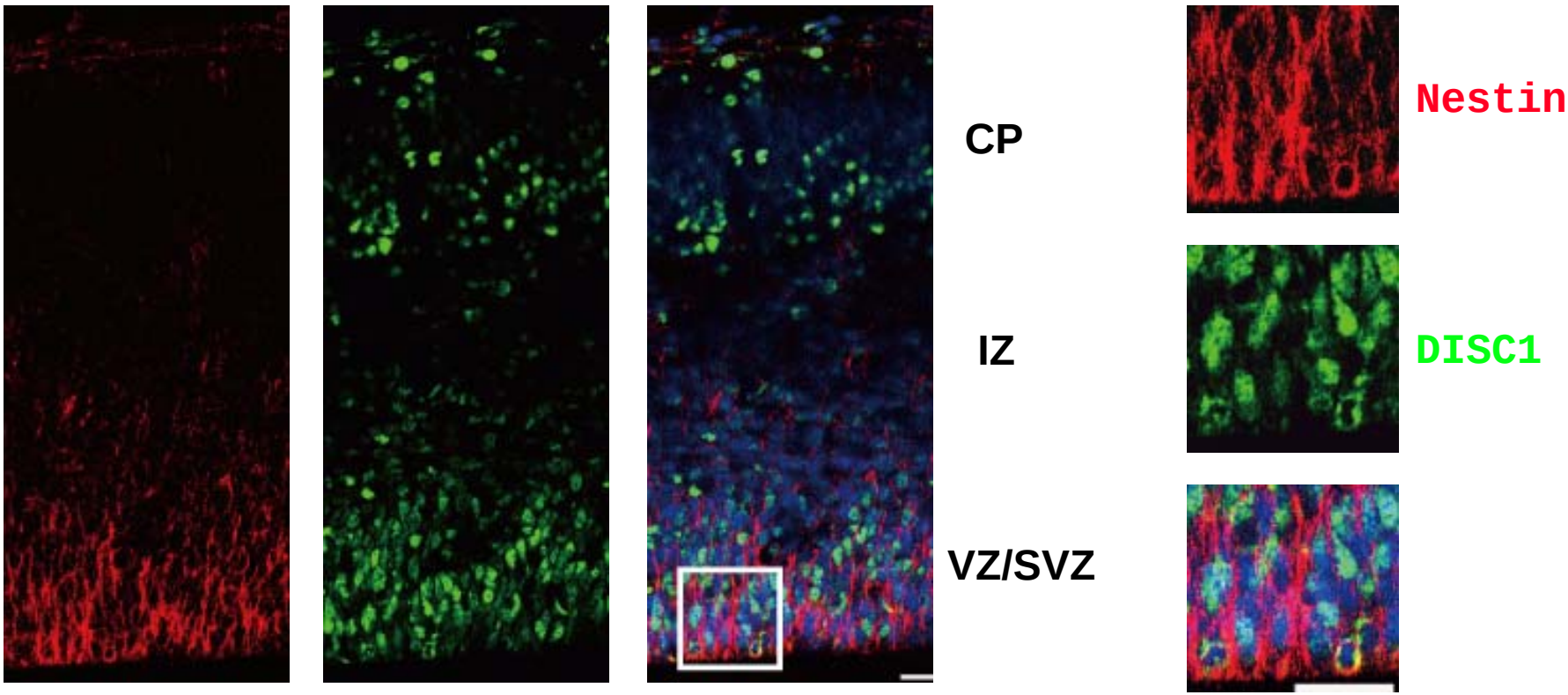
Summary of DISC1 mouse models

Group	Tg Mouse Features	Brain Morphology	Behavior			
			Locomotion	PPI	Working memory	Force swimming immobility
A Sawa et al	DN-DISC1 driven by CaMKII promoter	Enlarged ventricle	↑	↓	=	↑
C Ross et al	Tet-off inducible DISC1 truncation driven by CaMKII promoter	↓Brain volume	↑	N/A	↓	N/A
A Silva et al	DISC1 C-terminal fused to estrogen receptor and driven by CaMKII promoter	N/A	N/A	N/A	↓	↑
J Roder et al	Q31L – ENU mutagenesis	↓Brain volume	↑	↓	N/A	↑
	L100P – ENU mutagenesis	↓Brain volume	=	↓	N/A	=
J. Gogos et al	Exon 7/8 stop	Abnormal organization of newly born and mature neurons in DG	N/A	N/A	↓	N/A

Expression of DISC1 in the progenitor population during cortical development



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(Yingwei Mao)

DISC1 is prominently expressed in the dentate gyrus of adult brain

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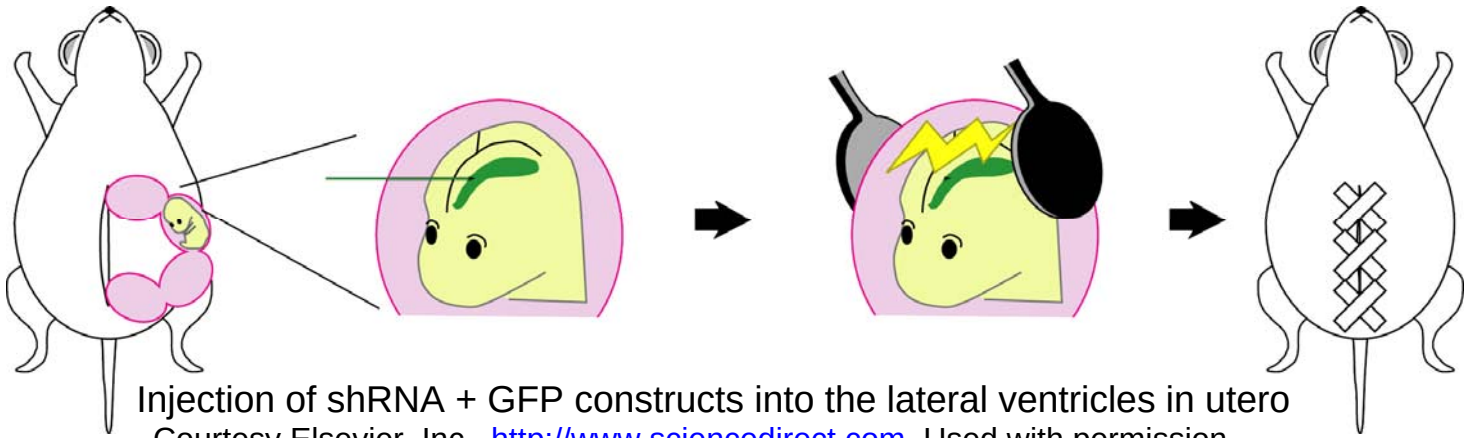
In utero electroporation-- Study of brain development



Injection of shRNA + GFP constructs into the lateral ventricles in utero

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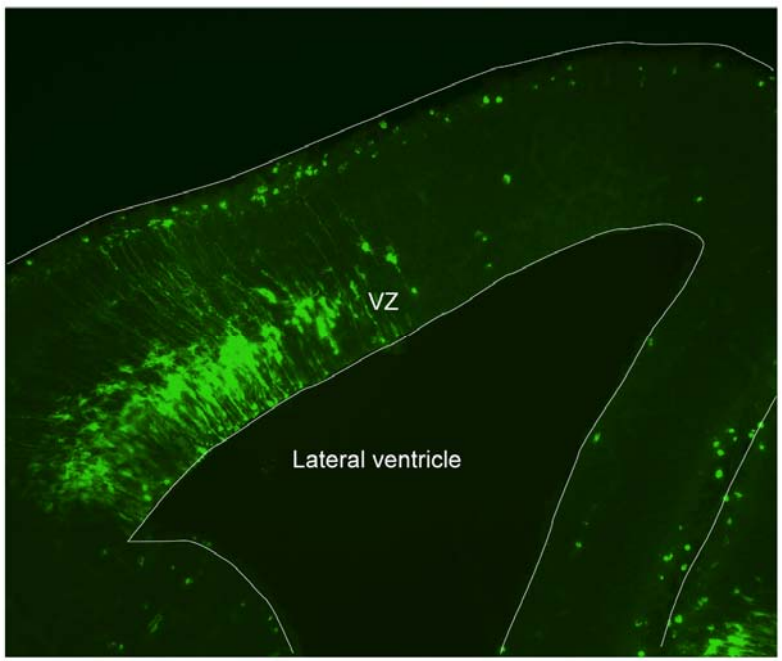

DISC1 is required for progenitor proliferation

1. DISC1 is abundantly expressed in the proliferative neural progenitors in embryonic and adult brains

2 DISC1 loss of function using the RNAi approach reduced the proliferation of the progenitors which was accompanied by increased neuronal differentiation

3. DISC1 gain of function by overexpression increased the proliferation of neural progenitors

Co-expression of DISC1 and β-catenin in cortical progenitors in the VZ

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Canonical Wnt signaling pathway

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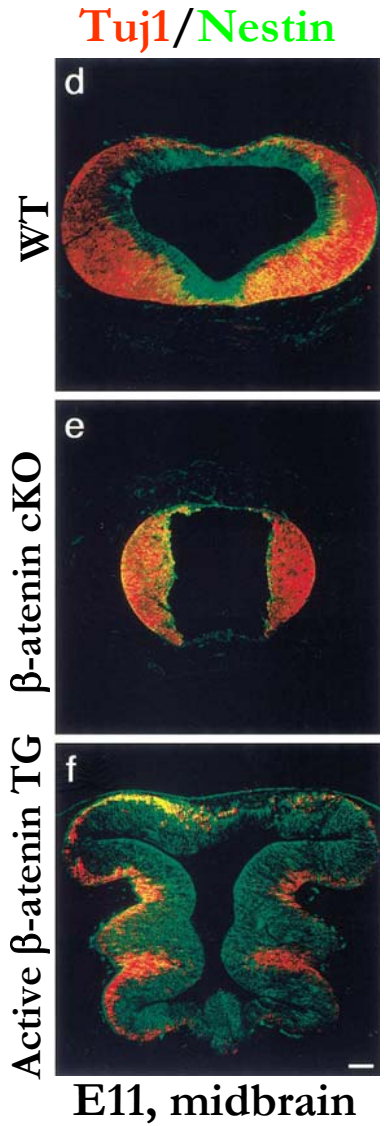
Wnt signaling in embryonic and adult neurogenesis

- Wnt proteins can act as stem cell growth factors (Nature. 2003;423:448)
- The Wnt pathway directs neuronal differentiation of cortical neural precursor cells. (Hirabayashi et al., Development. 2004)
- Transgenic mice expressing a stabilized -catenin in neural precursors develop enlarged brains with an expansion of the precursor population (Science 2002; 297: 365& Zechner et al. Developmental Biology, 2003).
- Wnt signalling regulates adult hippocampal neurogenesis (Lie et al, Nature, 2005)



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Summary

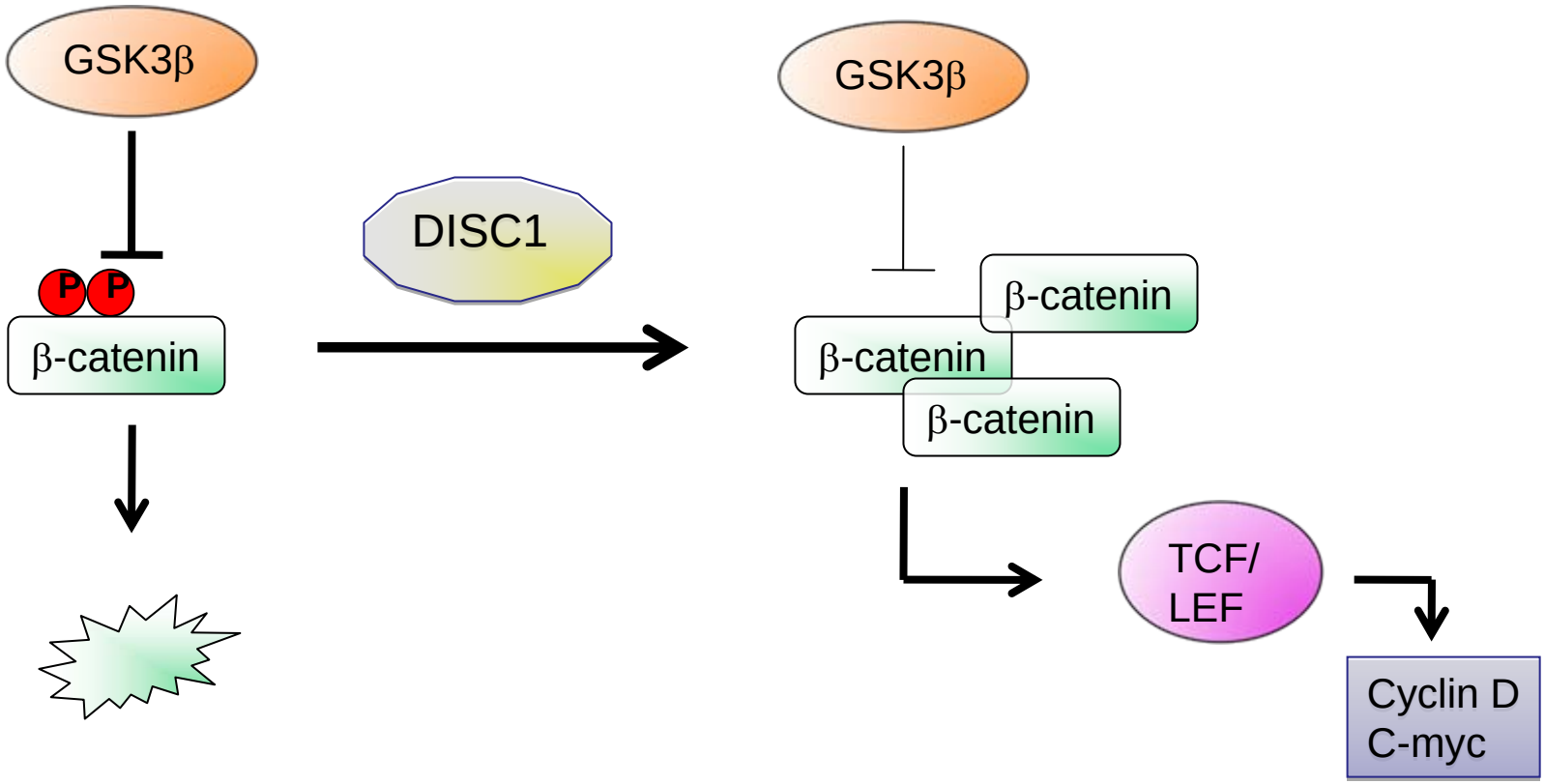
- **DISC1 is necessary for proper proliferation of brain cortical progenitors in embryonic development**

- **DISC1 positively regulates Wnt signaling in embryonic brain**

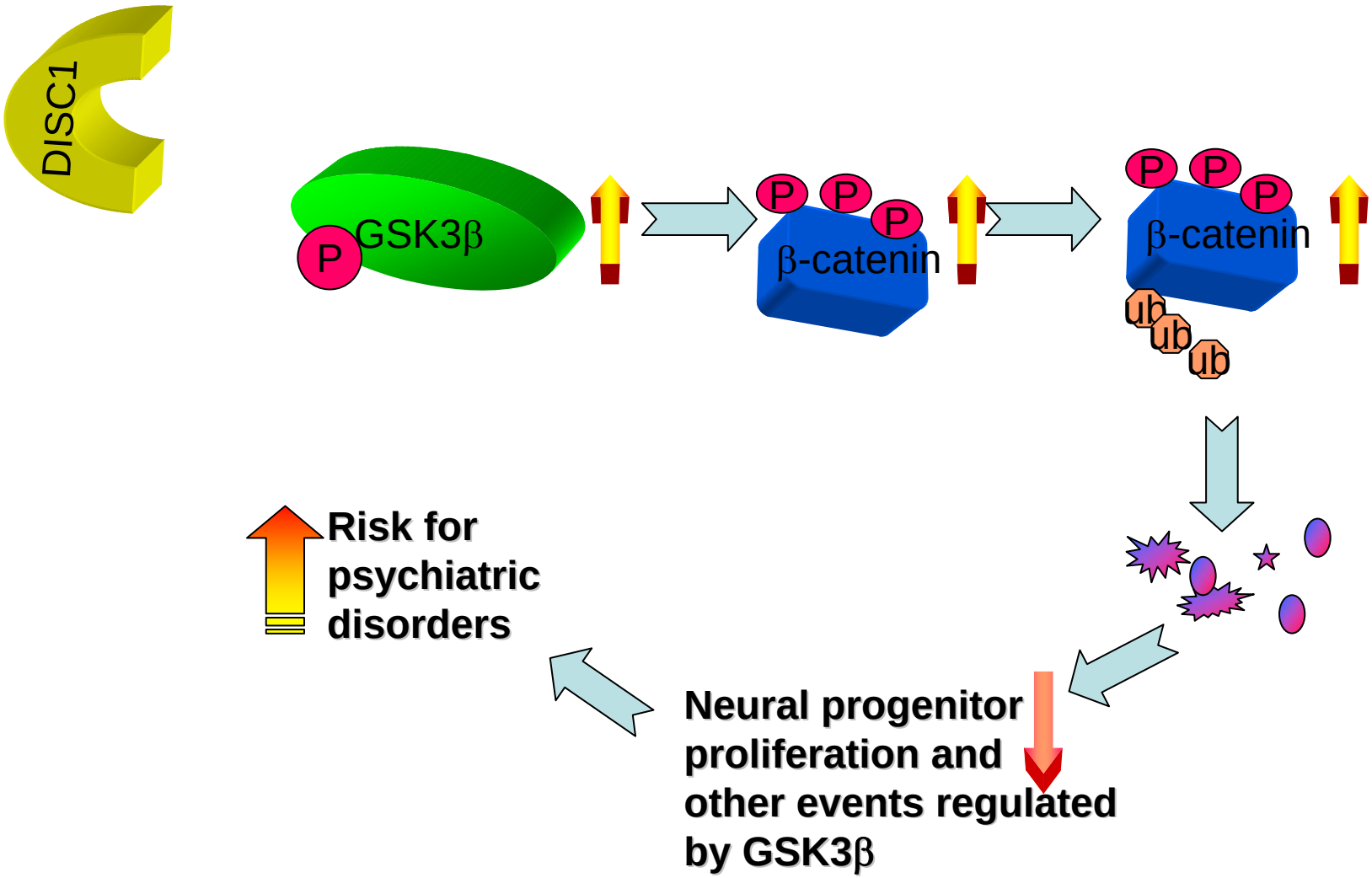
- **Stable β-catenin can rescue progenitor proliferation defect caused by DISC1 loss of function**

Hypothesis:

DISC1 acts upstream of β-catenin to regulate its abundance



Model


DISC1
GSK3β
P
β-catenin
P P P
β-catenin
P P P
ub
ub
ub
Risk for psychiatric disorders
Neural progenitor proliferation and other events regulated by GSK3β

Summary

- DISC1 regulates neurogenesis by promoting neural progenitor proliferation

- DISC1 stabilizes β-catenin levels and enhances transcriptional activity of TCF/LEF regulated genes

- DISC1 directly binds and inhibits GSK3β activity through its highly conserved N-terminal region

Lithium: the most commonly used treatment for bipolar disorder

- Lithium decreases the frequencies of manic and depression episodes
- Lithium in humans and animals ameliorate amphetamine induced phenotypes (euphoria/hyperactivity)
- Lithium is broadly considered a GSK3 inhibitor although it is reported to have other targets such as PI3K

A parallel function of DISC1 and lithium