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Similarities and Differences Between the Wnt and Reelin Pathways in the Forming Brain

Orly Reiner* and Tamar Sapir

Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot, Israel

Abstract

One of the key features in development is the reutilization of successful signaling pathways. Here, we emphasize the involvement of the Wnt pathway, one of the five kinds of signal transduction pathway predominating early embryonic development of all animals, in regulating the formation of brain structure. We discuss the interrelationships between the Wnt and reelin pathways in the regulation of cortical layering. We summarize data emphasizing key molecules, which, when mutated, result in abnormal brain development. This integrated view, which is based on conservation of pathways, reveals the relative position of participants in the pathway, points to control mechanisms, and allows raising testable working hypotheses. Nevertheless, although signaling pathways are highly conserved from flies to humans, the overall morphology is not. We propose that future studies directed at understanding of diversification will provide fruitful insights on mammalian brain formation.

Index Entries: Wnt; dishevelled; Presenilin; cdk5; Par-1; β-catenin; APP; lissencephaly; DCX; LIS1; JIP-1; JNK; reelin; ApoER2; phosphorylation; neuronal migration; brain development.

Introduction

Brain Development

Conservation and diversification, two seemingly contradictory actions, play a fundamental role in evolution. Signaling pathways are conserved throughout metazoa and it has been estimated that 17 kinds operate in all animals *(1)*. Our focus of interest is to identify and to understand the different signaling pathways that participate in the complex process of neocortical development and how they interact with each other. The functional complexity of the vertebrate cerebral cortex is facilitated by an intricate structural organization. In humans, formation of the brain occurs over months of prenatal development. Brain morphogenesis involves multiple steps, including cell proliferation, cell

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* Author to whom all correspondence and reprint requests should be addressed. E-mail: orly.reiner@weizmann.ac.il

fate identity, cell migration, and circuit formation following growth of axons and dendrites. The migratory pathway of individual neurons can be extremely long, especially in primates (reviewed in ref. *2*). Neurons in the neocortex are organized into six layers that share characteristic dendritic morphologies, axonal connections, and physiological properties (reviewed in refs. *3* and *4*). The neocortex is also regionalized into areas characterized by distinctive cytoarchitectonic features *(5)* that house specific cognitive, sensory, or motor functions *(6).* The axonal connections of neurons in different areas reflect their functional specificity. The six layers of the neocortex are formed during development mainly by inward-to-outward migration of neurons *(7)* born in the ventricular zone to the cortical plate *(8,9).*

Abnormal Brain Development

The most common disturbances of brain development affecting up to 4% of children cause functional deficits, leading, for instance, to epilepsy, mental retardation, behavioral disturbances, and functional psychosis *(10).* In addition, morphological abnormalities of the cortex account for a substantial fraction (5–15%) of epilepsy in adults *(11–13).* It has been estimated that harmful prenatal environmental events play a central role in the pathogenesis of neuronal migration defects in humans *(14–16).* Nevertheless, a portion of cortical malformations in humans are genetic in origin (reviews in refs. *17* and *18*). Abnormalities in the migration of neurons into the embryonic cortex lead in extreme cases to loss of normal convolutions of the cortex in humans, known as lissencephaly ("smooth brain"). There are two types of lissencephaly: type I or "classical lissencephaly," where four layers of abnormally positioned neurons are observed in the neocortex, and type II or "cobblestone" lissencephaly, where the cortex is unlayered *(19).* Mutations in two different genes might result in type I lissencephaly: *LIS1,* an autosomal gene located on chromosome 17p13.3 *(20),* and *doublecortin,* an X-linked gene *(21,22).* The most studied neuronal signaling pathway involved in neocortical layering is the reelin pathway (reviews in refs. *23* and *24*). In humans, reelin mutation is the cause of autosomal recessive lissencephaly with cerebellar hypoplasia *(25).*

Nevertheless, our information regarding this pathway is quite rudimentary. Therefore, the finding of similar and/or common components existing both in the reelin and Wnt (discussed next) pathways paved the route to outline these analogies in a more detailed fashion. As will be shown, there are multiple possibilities for crosstalk between the initially parallel pathways, and both play a significant role in brain development. Recent findings have allowed further strengthening of the proposed links.

The Reelin Pathway, Introductory Notes

The "basic" reelin pathway includes the large extracellular ligand reelin *(26–28),* two receptors belonging to the family of lipoprotein receptors (VLDLR and ApoER2) *(29–31),* and an intracellular molecule known as Dab1 *(25,32–34),* which needs to be phosphorylated *(35,36)* in order to properly propagate the reelin signal (for review, see ref. *24*). Mutations in the ligand, receptors, or the intracellular adaptor protein result in an indistinguishable phenotype known as the mouse "reeler" phenotype. Additional gene products have been implicated in this pathway and some of them will be discussed in this article. Reeler mice have multiple abnormal cell positioning in different areas of the central nervous system (CNS) (reviews in refs. *18* and *24*). In the cerebral cortex, the typical layered organization is not observed, and neurons born relatively late during corticogenesis reside in deep layers beneath the older neurons, thus inverted in comparison with the normal organization *(37,38).* Furthermore, the splitting of the preplate, which occurs when waves of newly born neurons migrate through the first-born generation of neurons, does not occur. So far, a direct ortholog of reelin has not been detected in

Drosophila or *C. elegans.* However, multiple members of the lipoprotein receptors and Dab1 exist both in fruit flies and worms (review in ref. *39*).

The Wnt Pathway, Introductory Notes

The Wnt pathway is highly conserved in evolution. Detailed analysis of components of this pathway revealed its function in numerous developmental roles. This includes roles as diverse as embryonic segmentation and patterning, gut patterning, nervous system development, formation and patterning of appendages, and stem cell proliferation. Most of the current information involves Wnt signaling through stabilization of β-catenin, which, in turn, enters the nucleus and controls gene expression (review in ref. *40*). Components of the Wnt signaling pathway are also regulated by other signals as well; thus, the realization of how different information from signaling pathways can be incorporated. A comprehensive "canonical Wnt/β-catenin pathway" including information from *Drosophila, Xenopus,* and *C. elegans,* has been composed (http://stke.sciencemag.org/cgi/cm/stkecm; CMP 5533). Nevertheless, an increasing amount of data has been accumulated for the non-β-catenin Wnt signaling "noncanonical Wnt pathway" (review in ref. *41*), where the best existing example is control of gastrulation movements. There are also indications for the participation of this pathway in processes as remote as cochlear hair cell morphology, heart induction, dorsoventral patterning, tissue separation, neuronal migration, and cancer. Potential signaling of the noncanonical Wnt pathway includes calcium flux, JNK (c-jun terminal kinase), and both small and heterotrimeric G proteins.

Ligands and Receptors

Wnt has been nominated as one of the five kinds of signal transduction pathway that predominate early embryonic development of all animals *(1).* Wnts compose a large group of secreted glycoproteins, including the wingless (wg) gene in *Drosophila* and numerous homologs across the phylogenetic tree (19 genes in human or mouse; http://www.stanford.edu/~rnusse/wntwindow.html), which serve as ligands, and their binding to membrane-coupled receptors initiate several signal transduction pathways. Wnt pathways are involved in the control of gene expression, cell behavior, cell adhesion, and cell polarity. In addition, they often operate in combination with other signaling pathways (reviews in refs. *42–44*). Multiple Wnt ligands are expressed in distinct patterns in the developing mouse *(45)* and human *(46)* brain. Loss-of-function mutations in individual Wnts cause deletions or malformations of distinct brain regions *(46–48).* In addition to the large repertoire of Wnt proteins, there are multiple extracellular proteins that participate in Wnt regulation as antagonists. The Wnts bind to Frizzled (Fz) seven-transmembrane-span receptors (for a schematic presentation of most of the molecules appearing in this section, *see* Fig. 1). Many of Wnt antagonists contain structural similarities to the receptor (review in ref. *49*). They can be generally divided into two different groups. The first group of proteins bind to Wnts and includes members of the sFRP (secreted Fz-related protein) family of proteins, WIF (Wnt inhibitory factor)-1, and Cer (Cerberus). The second group includes members of the Dkk (Dickkopf) family of proteins that binds to one subunit of the Wnt receptor complex. Both Dkk *(50)* and Cer *(51)* can inhibit brain formation in *Xenopus;* however, in the mouse Dkk *(52)* but not Cer *(53,54)* mutations inhibit normal head structures. Brain formation requires the simultaneous activity of additional secreted factors as bone morphogenetic proteins (BMPs) *(51,55,56).* In addition to Fz, LRP5/6 act as coreceptors of Wnt *(57,58).* In *Drosophila,* a mutation in the ortholog of LRP5/6 (the arrow gene) is phenotypically and molecularly indistinguishable from the wingless phenotype *(59).* The necessity of these coreceptors were demonstrated in the mouse, where deletion of a necessary chaperone for LRP5/6 resulted in disruption of embryonic polarity and mesoderm

differentiation *(60).* In addition, insertional mutagenesis of the mouse LRP6 locus resulted in developmental defects that were a striking composite of those caused by mutations in individual Wnt genes *(61).* Closer investigation of the brains of LRP6 mutants revealed reduced production of dentate granule neurons and abnormalities of the radial glial scaffolding (along which neurons migrate radially) in the forming dentate gyrus *(62).* LRP5/6 belong to the low-density lipoprotein receptor (LDLR) family of genes, whose products share a characteristic set of structural domains (review in ref. *57*). Interestingly, a related family member, LRP1, was shown to interact with the human Fz-1 (HFz1) and to downregulate the best studied (canonical) Wnt signaling pathway *(63).* Two additional family members, the VLDLR and ApoER2, have not been shown to have a direct connection with the Wnt signaling pathway; nevertheless, our working hypothesis is that these pathways converge downstream. As mentioned earlier, mutations in both of these receptors *(29–31),* in their ligand reelin *(26–28),* or in the intracellular signaling molecule Dab1, which binds to these receptors, result in abnormal lamination in mice and humans *(25,32–34).* Receptor clustering followed by Dab1 dimerization is essential for proper signaling *(64).* Because Dab1 not only binds to the intracellular domains of the VLDLR and apoER2 but also to those of LDLR, LRP, and LRP-2 *(31),* it could serve as a link between several signaling pathways.

Intracellular Signal Transduction

The Canonical Pathway and Beyond

The Wnt signaling is transmitted to the Dishevelled (Dsh) protein (review in refs. *44* and *65*). Dsh is an intriguing family of proteins (three Dsh-related genes in human and mouse) by virtue of the fact that it participates in at least three signaling pathways but yet is activated by similar Fz receptors. Within the cell, the Wnt pathway has several branches; the canonical Wnt/β-catenin pathway (review in ref. *42*), the planar cell polarity pathway (PCP) *(66–70),* and the Wnt/Ca^{2+} pathway *(71)* (for reviews, *see* refs. *41* and *72*). The best understood Wnt pathway is the "canonical" one. The Wnt-induced signaling leads to the activation of Dsh, which is regulated by several means. For example, the naked cuticle (nkd) gene attenuates Wnt signaling through a negative feedback loop mechanism (*see* Fig. 1) first discovered in *Drosophila (73,74)* and then in vertebrates *(75–77).* Fly and vertebrate Nkd proteins contain a putative calcium-binding motif, which interacts with Dsh *(74,76,78).* Dapper is an additional regulator of this pathway, acting at the level of Dsh. Depletion of maternal Dapper RNA from *Xenopus* embryos resulted in loss of notochord and head structures *(79).*

β-Catenin Stability

Dsh can suppress degradation of the β-catenin protein, enabling it to accumulate in the nucleus (Fig. 1). The suppression of degradation is through the inhibition of the so-called β-catenin destruction complex, a complex of proteins including APC, Axin, and GSK-3 that otherwise phosphorylates β-catenin, targeting it for destruction by the ubiquitin–proteasome pathway. These and other mechanisms allow Wnt ligands to both stabilize β-catenin and promote its entry into the nucleus where it recruits transactivators to HMG box DNA-binding proteins of the LEF/TCF family. Mice mutated for different components in this pathway exhibit different brain phenotypes. As mentioned previously, LRP6 mutants had obvious abnormalities in the hippocampus. These defects were more severe with the addition of a single Lef1 null allele (LRP6–/– Lef1–/+ mice) *(62).* Lef1–/– mice completely lack dendate gyrus hippocampal cells *(80).*

β-Catenin and the Brain

β-Catenin has two important cellular functions in a wide variety of tissues: (1) As a key component of the Wnt signaling pathway in embryonic development, it regulates gene tran-

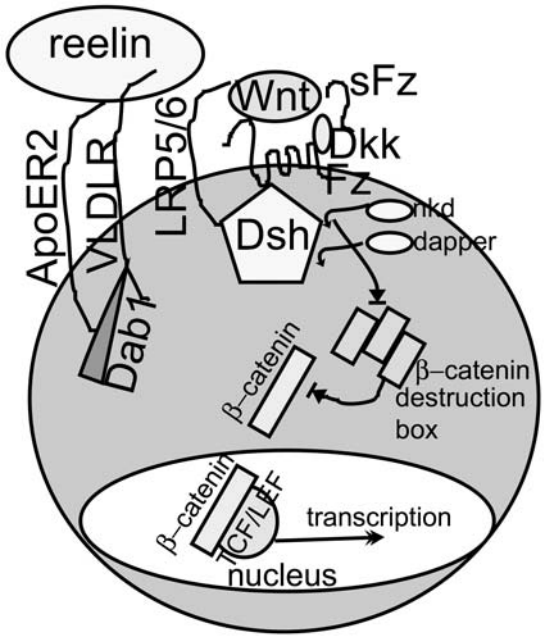

Fig. 1. Schematic presentation of the canonical Wnt pathway and the reelin pathway. The reelin signal is transmitted through binding to two receptors (ApoER2, VLDLR), that are structurally related to LRP5/6, one of the co-receptors to Wnt. Reelin signal mediates Dab1 dimerization and tyrosine phosphorylation. Wnt binds to frizzled (Fz) and LRP5/6. Secreted molecules as secreted frizzled (sFz) and Dickkopf (Dkk) can inhibit the Wnt pathway. Wnt signaling activates Dishevelled (Dsh), which can be modulated by naked cuticle (nkd), or dapper. Activation of Dsh suppresses the activity of the β-catenin destruction box, thus allowing the entry of β-catenin into the nucleus and TCF/LEF mediated transcription is initiated.

scription and (2) as a component of the cadherin/catenin complex, it helps to stabilize the actin cytoskeleton and to mediate cell adhesion (reviews in refs. *81–83*). There are numerous examples for the importance of β-catenin in the developing and adult brain. Most of the genetic loss-of-function studies of β-catenin *(84)* and gain-of-function *(85,86)* dramatically affect brain formation. Inactivation of β-catenin in the hippocampus and the cortex resulted in abnormalities in the hippocampus similar to those described in Lef1−/− mice and in severe abnormalities in the organization of the neuroepithelium of the cortex. This included disrupted interkinetic nuclear migration (movement of neurons within the ventricular zone, which is correlated with cell cycle), loss of adherens junctions, impaired radial migration of neurons toward superficial layers, and decreased cell proliferation after E15.5. At the newborn stage, a premature disassembly of the radial glial scaffold and increased numbers of astrocytes were found in the cortex *(87).* Furthermore, a function for β-catenin was demonstrated later in neuronal development in individual cells, where it regulated dendritic arborization *(88).* In hippocampal primary neurons lacking β-Catenin, abnormal synapse vesicle organization was observed *(89).* β-catenin is controlled by multiple molecules, which are not part of the canonical pathway (e.g., presenilin-1 [PS1]). The Alzheimer's disease-linked gene product PS1 is better known for its functions related to intramembrane proteolysis of amyloid precursor protein (APP) and Notch (review in ref. *90*). There are several examples of the intersection between the Notch and the Wnt pathways *(91–93).* PS1 interacts with β-catenin *(94–96),* thus acting as a scaffold, which negatively regulates β-catenin phosphorylation *(97–100).* This activity was found to be conserved from *Drosophila (101).* PS1 deficiency resulted in increased β-catenin stability in vitro and in vivo (the position of PS1 in the pathway is indicated in Fig. 2). In addition, PS1 was found to regulate the transcriptional activity of β-catenin, thus controlling β-catenin at two different levels *(102,103).* Interestingly, PS1−/− mice show brain abnormalities, with a phenotype resembling lissencephaly type II *(104–107).* The phenotype observed is probably the result of a combination of depletion of the neural progenitor cells with premature differentiation and migration to inappropriate targets. We raise the hypothesis that PS1 affects the Wnt/Ca^{2+} pathway as well as the canonical pathway, because it has been shown to have a role in the refilling mechanism for depleted intracellular calcium stores *(108,109).*

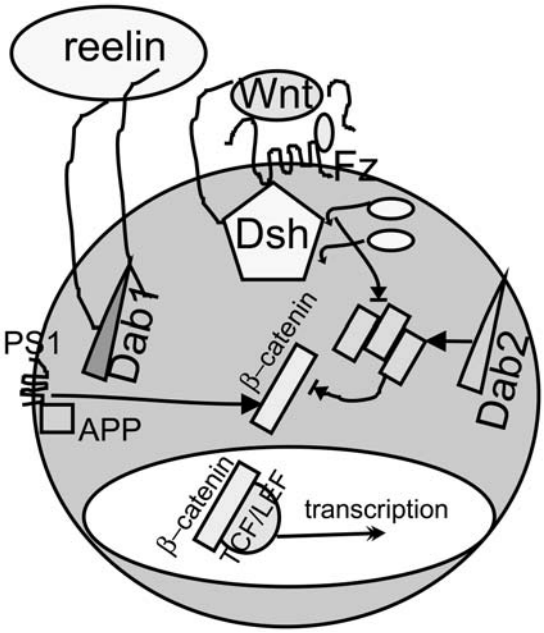

Fig. 2. The Wnt pathway is modulated by additional proteins, including Dab2, APP, and PS1. Dab2, which is similar to Dab1, can stabilize the β-catenin destruction box and inhibit Wnt signaling. Both Dab1 and Dab2 interact with APP, the processing of which is regulated by PS1. PS1 may also interact with β-catenin and negatively regulate its phosphorylation.

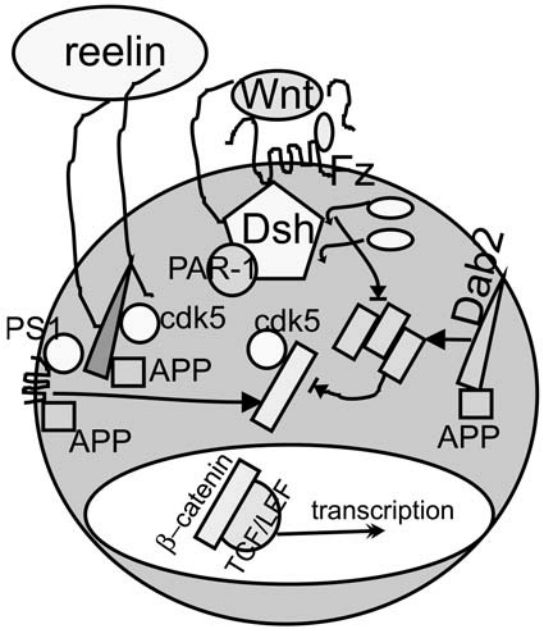

Fig. 3. Cdk5 and Par-1 phosphorylate multiple substrates found in the reelin and the Wnt pathway. Cdk5 interacts with and phosphorylates both PS1 and β-catenin. In addition, it phosphorylates Dab1. Par-1 is a kinase with activity important for cell polarity, regulates the Wnt pathway. Par-1 associates with Dsh, and is a positive regulator of the Wnt/β-catenin pathway.

β-Catenin Intracellular Localization

β-Catenin is regulated not only by transcription and degradation, but its intracellular localization is important to conduct its numerous functions. Recently, mutations in the gene ADP-ribosylation factor guanine nucleotide-exchange factor-2 (ARFGEF2) were implicated in microcephaly and periventricular heterotopia in humans *(122).* These mutations caused abnormal intracellular localization of β-catenin among many other proteins. It is indeed unclear whether this abnormal localization is causative for the observed brain phenotypes, but it might be a target of future investigations. Indeed, brain abnormalities as a result of expression of β-catenin gain of function *(85,86)* might be interpreted in part as abnormal intracellular localization of the protein.

Cdk5: A Kinase With Multiple Functions

An additional protein that has the potential of linking the Wnt/β-catenin and reelin pathways is cyclin-dependent kinase 5 (Cdk5). It has been shown to interact with and phosphorylate both PS1 and β-catenin *(108),* thus regulating the interaction between these two proteins (position indicated in Fig. 3). The activity of this enzyme links multiple components of cytoskeletal, membrane, and adhesion systems (reviewed in ref. *110*). Furthermore, Cdk5 activity is of great importance in the developing brain. Evidence of the involvement of Cdk5 in neuronal positioning came from analysis of mice with homozygous deletions in the genes encoding Cdk5 *(111)* or its neuronal activator p35 *(112);* these mice suffer from abnormal cortical layering. Cdk5 phosphory-

lates Dab1 independent of the reelin signaling pathway *(113,114).* In addition, Cdk5 phosphorylated NudeL *(115)* and DCX *(116),* both are LIS1 interacting proteins (described later), which interacts with reelin-induced phosphorylated Dab1 *(117).*

Dab2

Another modulator of the Wnt/β-catenin is Dab2, which has been proposed to function as a negative regulator of canonical Wnt signaling by stabilizing the β-catenin degradation complex *(118).* Dab2 is not only structurally similar to Dab1 *(119),* but they also interact with a common set of proteins including a GTPase activating protein *(120)* and APP *(119,121).* Therefore, Dab2 can serve as an additional link between the reelin and the Wnt pathway.

Par-1: An Additional Kinase That Might Link the Wnt and the Reelin Pathways

Phosphorylation and dephosphorylation are classical ways to control transient signaling pathways. Indeed, both kinases (as mentioned, cdk5) and phosphatases as PP2A *(123–128)* play an important role in control mechanisms of the Wnt pathway. An additional kinase that has been found to control the Wnt pathway in is Par-1 *(129).* Par-1 was associated with Dsh (indicated in Fig. 3), and its activity increased with the Wnt signal. Treating cells with Wnt increased endogenous Par-1 activity coincident with Dsh phosphorylation. Suppressing endogenous Par-1 function inhibited Wnt signaling through β-catenin in mammalian cells and *Xenopus* and *Drosophila* embryos. According to the results, Par-1 is a positive regulator of the Wnt/β-catenin pathway (canonical) and an inhibitor of the Wnt/JNK (noncannonical) pathway *(129).*

Par-1 is important for determination of cell polarity, first identified in *C. elegans (130),* then both in *Drosophila (131)* and mammalian cells *(132–134).* The mammalian homologs of Par-1, MARK, phosphorylated several microtubule-associated proteins (MAPs) *(132)* and probably participates in the initial polarity decisions of a differentiating neuron. Recently, DCX, a MAP *(135–137),* has been shown to be phosphorylated by MARK/Par-1 *(138),* by cdk5 *(116)* (Fig. 4), and by JNK *(139)* (Fig. 6).

Lissencephaly Associated Genes: The Reelin and Wnt Pathways

Mutations in *doublecortin* (DCX) cause X-linked lissencephaly and double cortex syndrome in humans *(21,22).* One of the sites phosphorylated in DCX by MARK/Par-1, the S47 residue, has been mutated in several lissencephaly patients *(21,22,138),* and overexpression of the mutated protein (S47R) abolished neurite outgrowth in PC12 cells *(140).* Interestingly, RNA interference (RNAi) of DCX has been shown to cause migrating neurons to adopt multipolar morphologies *(141),* suggesting that DCX plays a role in neuronal polarity. A large number of normal migrating neurons in the developing brain exhibit multipolar morphology, which might constitute a type of normal migration *(142,143);* therefore, the significance of these findings await further investigation. Nevertheless, these neurons do not migrate to their proper position. DCX localization at the neurite tips is maintained by phosphatase activity *(138).* Indeed, when neurons were treated with okadaic acid, which mainly inhibits the activity of PP2A, DCX was lost from most tips of the neurons. At present, it is unclear which sites are dephosphorylated by PP2A. As mentioned previously, PP2A plays a role also in the regulation of the Wnt pathway *(123–128).* DCX physically interacts with LIS1 (Fig. 4), as demonstrated using different biochemical assays *(144).* Moreover, genetic interactions between these two genes have been suggested. Mutations in LIS1 *(20),* or mutations in X-linked *DCX (21,22)* result in lissencephaly in human. In the mouse, *DCX* mutants exhibit a lamination defect in the hippocampus *(145)* identical to that described in Lis1–/+ mice *(146),* suggesting that the two gene products participate in the same pathway. Furthermore, recently a link between LIS1 and the reelin pathway has been suggested *(117),* and LIS1 has been shown to interact with

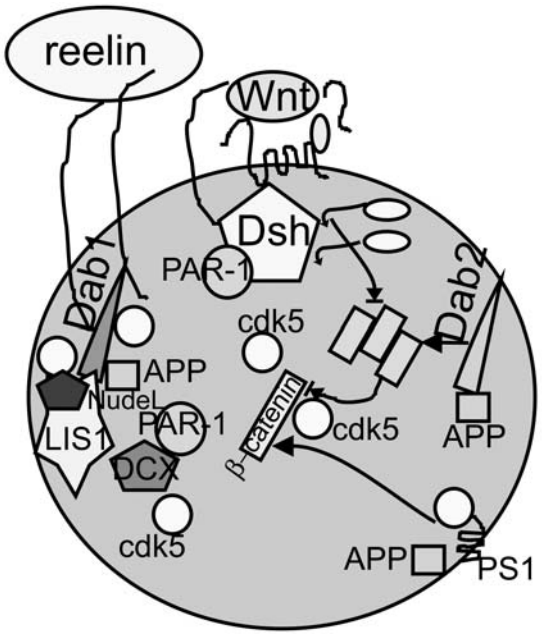

Fig. 4. Par-1 and Cdk5 both phosphorylate DCX, a microtubule-associated protein (MAP). Cdk5 also phosphorylates Nudel, a LIS1-interacting protein. In addition to Nudel, LIS1 interacts with DCX, and in a phospho-specific manner with Dab1, which is part of the reelin pathway.

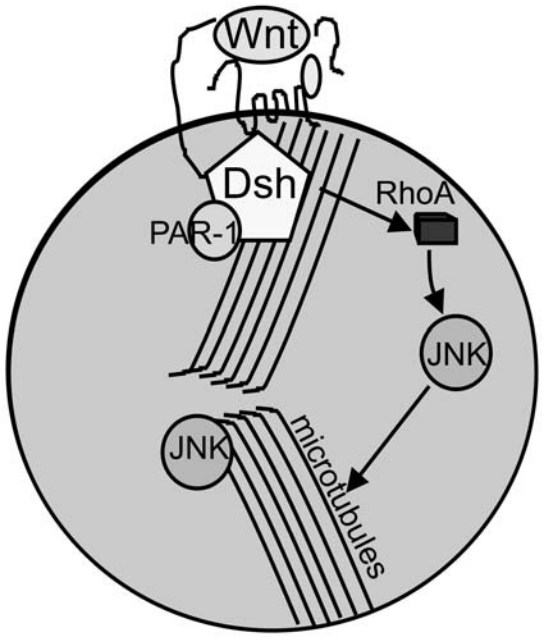

Fig. 5. The noncannonical JNK/Wnt pathway. In this pathway, Dsh is activated by Wnt at the membrane, it then activates downstream RhoA, which affects the cytoskeleton, and activates JNK, which phosphorylates additional downstream targets.

phosphorylated Dab1. LIS1 also interacts with Nudel and a similar protein mNudE *(115,147–152).* Nudel *(115)* and DCX *(116)* are both cdk5 substrates. Phosphorylation of DCX by cdk5 affected neuronal migration *(116).* Overexpression of DCX in cerebellar neurons resulted in faster migration, whereas a point mutation in S297 (the site phosphorylated by cdk5) or pharmacological inhibition of cdk5 abolished this effect.

The Noncanonical JNK/Wnt Pathway and More

The noncanonical JNK/Wnt pathway (Fig. 5) is best known for its involvement in changes of cell morphology and cell movement. It plays an essential role in the regulation of convergent extension movements in developing embryos *(66,67,70),* which is related to planar cell polarity in flies (reviews in refs. *41,153,* and *154*). The exact details of this alternative pathway are still controversial *(41).* In this pathway, the positioning of Dsh to the cell membrane is essential for its activity. In *Xenopus,* it is transported along microtubules, probably by a kinesin molecular motor *(155).* In *Drosophila,* it has been shown that Dsh activates downstream RhoA *(156–159),* which might affect the actin cytoskeleton, and activates JNK, which might phosphorylate additional downstream targets as MAPs and transcription factors in the nucleus. The same has been demonstrated in human cells and during *Xenopus* embryogenesis and also that RhoA activation requires a formin-domain protein, Daam1 *(160).* Inhibition or depletion of Daam1 prevents Wnt/Fz activation of Rho and of *Xenopus* gastrulation,

but not of β-catenin signaling. Formin homology (FH) proteins have been implicated in cell polarity from yeast to human *(161).* Furthermore, additional proteins, which are part of the pathway but are difficult to place within the cascade, are involved in neuronal migration. For instance, zebrafish Stbm *(161)* and Prickle1 *(66,162)* are required for the correct migration of hindbrain facial motor neurons from rhombomere 4 to rhombomeres 6 and 7.

The Importance of Scaffold Proteins

In processes involving cell polarity, the precise intracellular localization is crucial for proper function. To achieve this, spatial regulation protein kinases (as JNK) that form MAPK signaling modules might be organized into signaling complexes by binding to a common scaffold protein. The scaffold protein interacts with the proteins that compose the signaling module (review in ref. *163*). We detected an interaction between DCX and JNK Interacting Proteins, JIP1, or JIP2 *(139)* (Fig. 6). These proteins serve as scaffold JNK module and bind both activating and inhibitory components of this signaling pathway. Therefore, it is likely that proteins belonging to the noncanonical JNK/Wnt pathway will interact with JIP1/2 as well. JIP1/2 bind, among their multiple interacting proteins, JNK, MKK7, and members of the mixed-lineage kinase (MLK) group of MAP3K *(164,165).* Furthermore, both can bind the MAPK phosphatase MKP7 *(166).* The C-terminal region of JIP1/2 provides a link with a molecular motor, as its last 11 amino acids are essential for interaction with the microtubule-associated motor kinesin *(167),* and as mentioned earlier, Dsh is likely to be transported along microtubules by a kinesin *(155).* The interaction between the scaffold protein (known as Sunday driver) and the kinesin molecular motor is conserved and found in *Drosophila* and *C. elegans* as well *(168,169).* We demonstrated that the distribution of DCX to the proximal parts of neurites is dependent on its interaction with JIP1/2 and of the later with

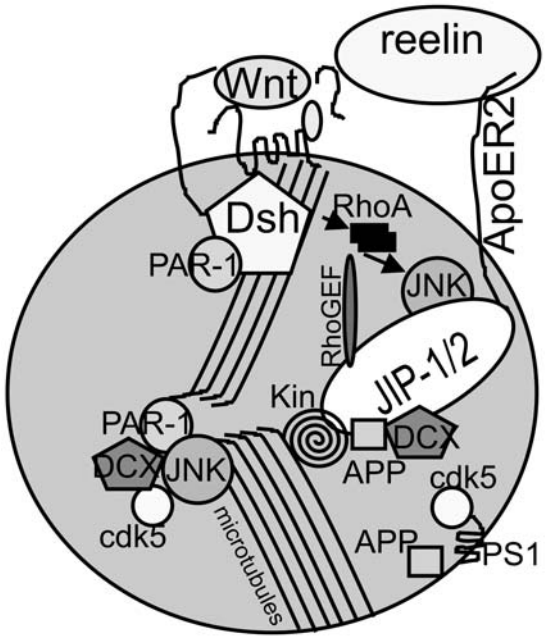

Fig. 6. JIP1/2 are scaffold proteins, which interact with the JNK signaling module and additional components. JIP1/2 interacts with ApoER2, one of reelin receptors. In addition, it interacts with RhoGEF, which belongs to the guanine nucleotide exchange factors family (GEF) active on Rho GTPases. It interacts with DCX, and in the same region, it interacts with APP. The C-terminal region of JIP1/2 is important for its interaction with the microtubule-dependent molecular motor kinesin (Kin).

kinesin *(139)* (review in ref. *170*). JIP1/2 are structurally similar and contain a SH3 domain and a PID (protein-interacting domain) domain in the C-terminal region *(164,165, 171–173).* The PID domain interacts with p190 RhoGEF *(174),* with APP *(175–177)* (which, in addition, interacts with Dab1, Dab2, and PS1), and with members of the LRP family, ApoER2 and Megalin (known also as LRP2) *(121,178).* ApoER2 as one of the receptors for reelin is of particular interest because it provides a link between DCX and the JNK pathway with the reelin pathway. As mentioned earlier, the same cortical layering phenotype is observed in cases of mutations in the extracellular protein

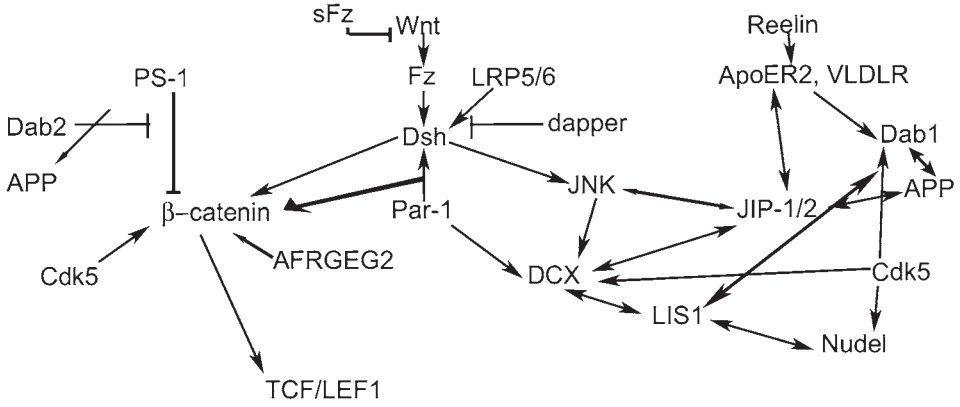

Fig. 7. Possible interrelationships among components of the β-catenin/Wnt pathway (left side), the JNK/Wnt pathway (middle), and the reelin pathway (right side). The extracellular bound proteins are located in the top of the scheme, followed by membrane-bound receptors and proteins. Intracellular components are drawn below and to the bottom nuclear proteins.

reelin *(26–28)*, in two of its multiple receptors (ApoER2 and VLDLR) *(29,30)*, or in the intracellular adaptor protein mDab1 *(25)*. JIP1/2 also interacts with DCX in the PID, and the same point mutation in the PID previously reported to affect the interaction of JIP-1 with RhoGEF *(174)* affected the interaction with DCX *(139)*. The JIP proteins also bind the Rac exchange factors Tiam1 and Ras-GRF1, although the interaction with JIP2 is significantly stronger than the interaction with JIP1 *(179)*.

Conclusions and Beyond

In this article, the overall similarities and possible crosstalks between the Wnt and the reelin pathways were highlighted (Fig. 7). In particular, the role of the different components of these pathways in brain development and neuronal migration were emphasized. The similarities are less obvious at the ligand levels. Structural similarities are observed at the receptor levels and LRP5/6, ApoER2, and VLDLR belong to the same gene family. Likewise, Dab1 and Dab2 are close homologs. The interconnectivity of these pathways is apparent downstream. Several of the proteins play a role in both pathways. For example, Par-1, which has a significant role in regulation of the Wnt pathway, phosphorylates DCX. Cdk5 phosphorylates multiple substrates, which are part of both pathways. JIP-1/2 as a JNK scaffold protein and as a RhoGEF-binding protein is likely to take part in the JNK/Wnt pathway and to link it to the reelin pathway through interactions with ApoER2, DCX, and APP. A portion of the several possible crosstalk possibilities is shown in the general scheme (Fig. 7). Based on the fact that successful pathways are reutilized multiple times, it is evident that the Wnt pathway plays an important role not only during early developmental events but also in regulating the formation of the proper brain structure (*see also* reviews in refs. *43* and *180*). Indeed, Wnt signaling has been shown to be important not only for synaptogenesis, axon, and dendrite development *(88,181)* but also for maintenance of the integrity of the nervous system (as exemplified in frizzled 4 mutant mice) *(182)*.

We have emphasized the striking similarities in conservation of the relevant signaling pathways; nevertheless, the overall similarity between the "brain" of the fly and the human brain is minimal. This notion was already

raised by Darwin in his "principle of divergence" *(183):*

> I have overlooked one problem of great importance; and it is astonishing to me how I could have overlooked it and its solution. This problem is the tendency in organic beings descended from the same stock to diverge in character as they become modified. That they have diverged greatly is obvious from the manner in which species of all kinds can be classed under genera, genera under families, families under sub-order and so forth… The solution occurred to me long after I had come to Down. The solution, as I believe, is that the modified offspring of all dominant and increasing forms tend to become adapted to many and highly diversified places in the economy of nature.

The understanding of the species diversification is still lacking, although possible driving forces taking part in this process were termed as evolvability (discussed briefly below) *(184).* Evolvability is an organism's capacity to generate heritable phenotypic variation. Core biological processes are highly conserved, probably the result of recurrent selection. The control mechanisms involved (mainly inhibitory) are usually subject to a high degree of variance, making this a flexible component. In addition, weak coupling of processes, such as signal transduction pathways, facilitates each component's accommodation to novelty. Furthermore, many biological systems are highly flexible and might lead to a wide repertoire of states, which might be stabilized in different modes. These systems are not only flexible but highly robust and, as such, are able to accommodate changes. An additional factor playing a role in evolvability is compartmentalization, at the gene expression level or the body axis level. Consequently, any specific change (or mutation) could be limited to a particular area, and not spread out throughout the whole animal. Thus, following the conservation and similarities, understanding of the dissimilarity might be an appealing and ample line of research.

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References

1. Gerhart J. (1999) 1998 Warkany lecture: signaling pathways in development. *Teratology* **60,** 226–239.
2. Rakic P. (1997) Intra- and extracellular control of neuronal migration: relevance to cortical malformations, in *Normal and Abnormal Development of Cortex,* Galaburn A.M. and Christen Y., eds., Springer-Verlag, New York, pp. 81–98.
3. McConnell S.K. (1988) Development and decision-making in the mammalian cerebral cortex. *Brain Res. Rev.* **13,** 1–23.
4. McConnell S.K. (1991) The generation of neuronal diversity in the central nervous system. *Annu. Rev. Neurosci.* **14,** 269–300.
5. Brodmann K. (1909) *Lokalisationslehre der Groshirnrinde in ihren Principen dargestellt aus Grund des Zellen baue.* Barth, Leipzig.
6. Payne B.R., Lomber S.G., Villa A.E., and Bullier J. (1996) Reversible deactivation of cerebral network components. *Trends Neurosci.* **19,** 535–542.
7. Takahashi T., Goto T., Miyama S., Nowakowski R.S., and Caviness V.S., Jr. (1999) Sequence of neuron origin and neocortical laminar fate: relation to cell cycle of origin in the developing murine cerebral wall [In Process Citation]. *J. Neurosci.* **19,** 10,357–10,371.
8. Angevine J.B. and Sidman R.L. (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* **192,** 766–768.

9. Rakic P. (1974) Neurons in Rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. *Science* **183,** 425–427.
10. Office of Population Censuses and Surveys. (1989) *The Prevalence of Disability Among Children.* Her Majesty's Stationery Office, London.
11. Hardiman O., Burke T., Phillips J., et al. (1988) Microdysgenesis in resected temporal neocortex: incidence and clinical significance in focal epilepsy. *Neurology* **38,** 1041–1047.
12. Brodtkorb E., Nilsen G., Smevik O., and Rinck P.A. (1992) Epilepsy and anomalies of neuronal migration: MRI and clinical aspects. *Acta Neurol. Scand.* **86,** 24–32.
13. Hauser W.A., Annegers J.F., and Kurland L.T. (1993) Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935–1984. *Epilepsia* **34,** 453–468.
14. Palmini A., Andermann E., and Andermann F. (1994) Prenatal events and genetic factors in epileptic patients with neuronal migration disorders. *Epilepsia* **35,** 965–973.
15. Pratt O.E. and Doshi R. (1984) Range of alcohol-induced damage in the developing central nervous system. *CIBA Found. Symp.* **105,** 142–156.
16. Clarren S.K., Alvord E.C., Jr., Sumi S.M., Streissguth A.P., and Smith D.W. (1978) Brain malformations related to prenatal exposure to ethanol. *J. Pediatr.* **92,** 64–67.
17. Walsh C.A. (1999) Genetic malformations of the human cerebral cortex. *Neuron* **23,** 19–29.
18. Gupta A., Tsai L.H., and Wynshaw-Boris A. (2002) Life is a journey: a genetic look at neocortical development. *Nat. Rev. Genet.* **3,** 342–355.
19. Barkovich A.J., Kuzniecky R.I., Dobyns W.B., et al. (1996) A classification scheme for malformations of cortical development. *Neuropediatrics* **27,** 59–63.
20. Reiner O., Carrozzo R., Shen Y., et al. (1993) Isolation of a Miller–Dieker lissencephaly gene containing G protein β-subunit-like repeats. *Nature* **364,** 717–721.
21. des Portes V., Pinard J.M., Billuart P., et al. (1998) A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar hetrotropia and lissencephaly syndrome. *Cell* **92,** 51–61.
22. Gleeson J.G., Allen K.M., Fox J.W., et al. (1998) *doublecortin,* a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell* **92,** 63–72.
23. Tissir F. and Goffinet A.M. (2003) Reelin and brain development. *Nat. Rev. Neurosci.* **4,** 496–505.
24. Rice D.S. and Curran T. (2001) Role of the reelin signaling pathway in central nervous system development. *Annu. Rev. Neurosci.* **24,** 1005–1039.
25. Hong S.E., Shugart Y.Y., Huang D.T., et al. (2000) Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat. Genet.* **26,** 93–96.
26. D'Arcangelo G., Miao G.G., Chen S.C., Soares H.D., Morgan J.I., and Curran T. (1995) A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* **374,** 719–723.
27. Ogawa M., Miyata T., Nakajiman K., et al. (1995) The *reeler* gene-associated antigen on Cajal–Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* **14,** 899–912.
28. Hirotsune S., Takahara T., Sasaki N., et al. (1995) The reeler gene encodes a protein with an EGF-like motif expressed by pioneer neurons. *Nat. Genet.* **10,** 77–84.
29. D'Arcangelo G., Homayouni R., Keshvara L., Rice D.S., Sheldon M., and Curran T. (1999) Reelin is a ligand for lipoprotein receptors. *Neuron* **24,** 471–479.
30. Hiesberger T., Trommsdorff M., Howell B.W., et al. (1999) Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* **24,** 481–489.
31. Trommsdorff M., Gotthardt M., Hiesberger T., et al. (1999) Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* **97,** 689–701.
32. Howell B.W., Gertler F.B., and Cooper J.A. (1997) Mouse disabled (mDab1): a Src binding protein implicated in neuronal development. *EMBO J.* **16,** 121–132.
33. Ware M.L., Fox J.W., Gonzalez J.L., et al. (1997) Aberrant splicing of a mouse *disabled* homolog, *mdab1,* in the *scrambler* mouse. *Neuron* **19,** 239–249.
34. Sheldon M., Rice D.S., D'Arcangelo G., et al. (1997) *Scrambler* and *yotari* disrupt the *disabled* gene and produce a *reeler*-like phenotype in mice. *Nature* **389,** 730–733.

35. Howell B.W., Herrick T.M., and Cooper J.A. (1999) Reelin-induced tryosine phosphorylation of disabled 1 during neuronal positioning. *Genes Dev.* **13,** 643–648.
36. Howell B.W., Herrick T.M., Hildebrand J.D., Zhang Y., and Cooper J.A. (2000) Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. *Curr. Biol.* **10,** 877–885.
37. Caviness V.S., Jr. and Sidman R.L. (1973) Time of origin or corresponding cell classes in the cerebral cortex of normal and reeler mutant mice: an autoradiographic analysis. *J. Comp. Neurol.* **148,** 141–151.
38. Caviness V.S., Jr. and Sidman R.L. (1973) Retrohippocampal, hippocampal and related structures of the forebrain in the reeler mutant mouse. *J. Comp. Neurol.* **147,** 235–254.
39. Herz J. and Bock H.H. (2002) Lipoprotein receptors in the nervous system. *Annu. Rev. Biochem.* **71,** 405–434.
40. Nelson W.J. and Nusse R. (2004) Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* **303,** 1483–1487.
41. Veeman M.T., Axelrod J.D., and Moon R.T. (2003) A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev. Cell* **5,** 367–377.
42. Moon R.T., Bowerman B., Boutros M., and Perrimon N. (2002) The promise and perils of Wnt signaling through beta-catenin. *Science* **296,** 1644–1646.
43. Patapoutian A. and Reichardt L.F. (2000) Roles of Wnt proteins in neural development and maintenance. *Curr. Opin. Neurobiol.* **10,** 392–399.
44. Huelsken J. and Behrens J. (2002) The Wnt signalling pathway. *J. Cell Sci.* **115,** 3977–3978.
45. Grove E.A., Tole S., Limon J., Yip L., and Ragsdale C.W. (1998) The hem of the embryonic cerebral cortex is defined by the expression of multiple Wnt genes and is compromised in Gli3-deficient mice. *Development* **125,** 2315–2325.
46. Lee S.M., Tole S., Grove E., and McMahon A.P. (2000) A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* **127,** 457–467.
47. Thomas K.R., Musci T.S., Neumann P.E., and Capecchi M.R. (1991) Swaying is a mutant allele of the proto-oncogene Wnt-1. *Cell* **67,** 969–976.
48. McMahon A.P. and Bradley A. (1990) The *Wnt-1 (int-1)* proto-oncogene is required for development of a large region of the mouse brain. *Cell* **62,** 1073–1085.
49. Kawano Y. and Kypta R. (2003) Secreted antagonists of the Wnt signalling pathway. *J. Cell Sci.* **116,** 2627–2634.
50. Glinka A., Wu W., Delius H., Monaghan A.P., Blumenstock C. and Niehrs C. (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* **391,** 357–362.
51. Piccolo S., Agius E., Leyns L., et al. (1999) The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. *Nature* **397,** 707–710.
52. Mukhopadhyay M., Shtrom S., Rodriguez-Esteban C., et al. (2001) Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. *Dev. Cell* **1,** 423–434.
53. Stanley E.G., Biben C., Allison J., et al. (2000) Targeted insertion of a lacZ reporter gene into the mouse Cer1 locus reveals complex and dynamic expression during embryogenesis. *Genesis* **26,** 259–264.
54. Shawlot W., Min Deng J., Wakamiya M., and Behringer R.R. (2000) The cerberus-related gene, Cerr1, is not essential for mouse head formation. *Genesis* **26,** 253–258.
55. Glinka A., Wu W., Onichtchouk D., Blumenstock C., and Niehrs C. (1997) Head induction by simultaneous repression of Bmp and Wnt signalling in *Xenopus. Nature* **389,** 517–519.
56. Niehrs C., Kazanskaya O., Wu W., and Glinka A. (2001) Dickkopf1 and the Spemann–Mangold head organizer. *Int. J. Dev. Biol.* **45,** 237–240.
57. Schneider W.J. and Nimpf J. (2003) LDL receptor relatives at the crossroad of endocytosis and signaling. *Cell. Mol. Life Sci.* **60,** 892–903.
58. Mao B., Wu W., Li Y., et al. (2001) LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature* **411,** 321–325.
59. Wodarz A. and Nusse R. (1998) Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14,** 59–88.
60. Hsieh J.C., Lee L., Zhang L., et al. (2003) Mesd encodes an LRP5/6 chaperone essential for specification of mouse embryonic polarity. *Cell* 112, 355–367.
61. Pinson K.I., Brennan J., Monkley S., Avery B.J., and Skarnes W.C. (2000) An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* **407,** 535–538.
62. Zhou C.J., Zhao C., and Pleasure S.J. (2004) Wnt signaling mutants have decreased dentate

granule cell production and radial glial scaffolding abnormalities. *J. Neurosci.* **24,** 121–126.
63. Zilberberg A., Yaniv A., and Gazit A. (2004) The low-density lipoprotein receptor-1, LRP1, interacts with the human frizzled-1 (HFz1) and downregulates the canonical Wnt signaling pathway. *J. Biol. Chem.* **279,** 17,535–17,542.
64. Strasser V., Fasching D., Hauser C., et al. (2004) Receptor clustering is involved in Reelin signaling. *Mol. Cell. Biol.* **24,** 1378–1386.
65. Boutros M. and Mlodzik M. (1999) Dishevelled: at the crossroads of divergent intracellular signaling pathways. *Mech. Dev.* **83,** 27–37.
66. Veeman M.T., Slusarski D.C., Kaykas A., Louie S.H., and Moon R.T. (2003) Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. *Curr. Biol.* **13,** 680–685.
67. Wiggan O. and Hamel P.A. (2002) Pax3 regulates morphogenetic cell behavior in vitro coincident with activation of a PCP/noncanonical Wnt-signaling cascade. *J. Cell Sci.* **115,** 531–541.
68. Li L., Yuan H., Xie W., et al. (1999) Dishevelled proteins lead to two signaling pathways. Regulation of LEF-1 and c-Jun N-terminal kinase in mammalian cells. *J. Biol. Chem.* **274,** 129–134.
69. Tomlinson A., Strapps W.R., and Heemskerk J. (1997) Linking Frizzled and Wnt signaling in *Drosophila* development. *Development* **124,** 4515–4521.
70. Yamanaka H., Moriguchi T., Masuyama N., et al. (2001) JNK functions in the non-canonical Wnt pathway to regulate convergent extension movements in vertebrates. *EMBO Rep.* **19,** 19.
71. Slusarski D.C., Yang-Snyder J., Busa W.B., and Moon R.T. (1997) Modulation of embryonic intracellular Ca2+ signaling by Wnt-5A. *Dev. Biol.* **182,** 114–120.
72. Kuhl M., Sheldahl L.C., Park M., Miller J.R., and Moon R.T. (2000) The Wnt/Ca2+ pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet.* **16,** 279–283.
73. Zeng W., Wharton K.A., Jr., Mack J.A., et al. (2000) Naked cuticle encodes an inducible antagonist of Wnt signalling. *Nature* **403,** 789–795.
74. Rousset R., Mack J.A., Wharton K.A., Jr., et al. (2001) Naked cuticle targets dishevelled to antagonize Wnt signal transduction. *Genes Dev.* **15,** 658–671.
75. Yan D., Wallingford J.B., Sun T.Q., et al. (2001) Cell autonomous regulation of multiple Dishevelled-dependent pathways by mammalian Nkd. *Proc. Natl. Acad. Sci. USA* **98,** 3802–3807.
76. Wharton K.A., Jr., Zimmermann G., Rousset R., and Scott M.P. (2001) Vertebrate proteins related to *Drosophila* Naked Cuticle bind Dishevelled and antagonize Wnt signaling. *Dev. Biol.* **234,** 93–106.
77. Katoh M. (2001) Molecular cloning, gene structure, and expression analyses of NKD1 and NKD2. *Int. J. Oncol.* **19,** 963–969.
78. Rousset R., Wharton K.A., Jr., Zimmermann G., and Scott M.P. (2002) Zinc-dependent interaction between dishevelled and the Drosophila Wnt antagonist naked cuticle. *J. Biol. Chem.* **277,** 49,019–49,026.
79. Cheyette B.N., Waxman J.S., Miller J.R., et al. (2002) Dapper, a Dishevelled-associated antagonist of beta-catenin and JNK signaling, is required for notochord formation. *Dev. Cell* **2,** 449–461.
80. Galceran J., Miyashita-Lin E.M., Devaney E., Rubenstein J.L., and Grosschedl R. (2000) Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. *Development* **127,** 469–482.
81. Cadigan K.M. and Nusse R. (1997) Wnt signaling: a common theme in animal development. *Genes Dev.* **11,** 3286–3305.
82. Polakis P. (2000) Wnt signaling and cancer. *Genes Dev.* **14,** 1837–1851.
83. Bienz M. and Clevers H. (2000) Linking colorectal cancer to Wnt signaling. *Cell* **103,** 311–320.
84. Brault V., Moore R., Kutsch S., et al. (2001) Inactivation of the beta-catenin gene by Wnt1-Cre-mediated deletion results in dramatic brain malformation and failure of craniofacial development. *Development* **128,** 1253–1264.
85. Zechner D., Fujita Y., Hulsken J., et al. (2003) Beta-catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. *Dev. Biol.* **258,** 406–418.
86. Chenn A. and Walsh C.A. (2003) Increased neuronal production, enlarged forebrains and cytoarchitectural distortions in beta-catenin overexpressing transgenic mice. *Cereb. Cortex* **13,** 599–606.
87. Machon O., van den Bout C.J., Backman M., Kemler R., and Krauss S. (2003) Role of beta-catenin in the developing cortical and hippocampal neuroepithelium. *Neuroscience* **122,** 129–143.

88. Yu X. and Malenka R.C. (2003) Beta-catenin is critical for dendritic morphogenesis. *Nat. Neurosci.* **6,** 1169–1177.
89. Bamji S.X., Shimazu K., Kimes N., et al. (2003) Role of beta-catenin in synaptic vesicle localization and presynaptic assembly. *Neuron* **40,** 719–731.
90. Selkoe D. and Kopan R. (2003) Notch and Presenilin: regulated intramembrane proteolysis links development and degeneration. *Annu. Rev. Neurosci.* **26,** 565–597.
91. Ruel L., Bourouis M., Heitzler P., Pantesco V., and Simpson P. (1993) *Drosophila* shaggy kinase and rat glycogen synthase kinase-3 have conserved activities and act downstream of Notch. *Nature* **362,** 557–560.
92. Couso J.P. and Martinez Arias A. (1994) Notch is required for wingless signaling in the epidermis of *Drosophila. Cell* **79,** 259–272.
93. Axelrod J.D., Matsuno K., Artavanis-Tsakonas S., and Perrimon N. (1996) Interaction between Wingless and Notch signaling pathways mediated by dishevelled. *Science* **271,** 1826–1832.
94. Murayama M., Tanaka S., Palacino J., et al. (1998) Direct association of presenilin-1 with beta-catenin. *FEBS Lett.* **433,** 73–77.
95. Yu G., Chen F., Levesque G., et al. (1998) The presenilin 1 protein is a component of a high molecular weight intracellular complex that contains beta-catenin. *J. Biol. Chem.* **273,** 16,470–16,475.
96. Georgakopoulos A., Marambaud P., Efthimiopoulos S., et al. (1999) Presenilin-1 forms complexes with the cadherin/catenin cell-cell adhesion system and is recruited to intercellular and synaptic contacts. *Mol. Cell* **4,** 893–902.
97. Kang D.E., Soriano S., Xia X., et al. (2002) Presenilin couples the paired phosphorylation of beta-catenin independent of axin: implications for beta-catenin activation in tumorigenesis. *Cell* **110,** 751–762.
98. Soriano S., Kang D.E., Fu M., et al. (2001) Presenilin 1 negatively regulates beta-catenin/T cell factor/lymphoid enhancer factor-1 signaling independently of beta-amyloid precursor protein and notch processing. *J. Cell Biol.* **152,** 785–794.
99. Kang D.E., Soriano S., Frosch M.P., et al. (1999) Presenilin 1 facilitates the constitutive turnover of beta-catenin: differential activity of Alzheimer's disease-linked PS1 mutants in the beta-catenin-signaling pathway. *J. Neurosci.* **19,** 4229–4237.
100. Singh N., Talalayeva Y., Tsiper M., et al. (2001) The role of Alzheimer's disease-related presenilin 1 in intercellular adhesion. *Exp. Cell Res.* **263,** 1–13.
101. Noll E., Medina M., Hartley D., Zhou J., Perrimon N., and Kosik K.S. (2000) Presenilin affects arm/beta-catenin localization and function in Drosophila. *Dev. Biol.* **227,** 450–464.
102. Palacino J.J., Murphy M.P., Murayama O., et al. (2001) Presenilin 1 regulates beta-catenin-mediated transcription in a glycogen synthase kinase-3-independent fashion. *J. Biol. Chem.* **276,** 38,563–38,569.
103. Killick R., Pollard C.C., Asuni A.A., et al. (2001) Presenilin 1 independently regulates beta-catenin stability and transcriptional activity. *J. Biol. Chem.* **276,** 48,554–48,561.
104. Hartmann D., Strooper B.D., and Saftig P. (1999) Presenilin-1 deficiency leads to loss of Cajal–Retzius neurons and cortical dysplasia similar to human type 2 lissencephaly. *Curr. Biol.* **9,** 719–727.
105. Shen J., Bronson R.T., Chen D.F., Selkoe D.J., and Tonegawa S. (1997) Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell* **89,** 629–639.
106. Yang X., Handler M., and Shen J. (2000) Role of presenilin-1 in murine neural development. *Ann. NY Acad. Sci.* **920,** 165–170.
107. Louvi A., Sisodia S.S., and Grove E.A. (2004) Presenilin 1 in migration and morphogenesis in the central nervous system. *Development* **131,** 3093–3105.
108. Leissring M.A., Akbari Y., Fanger C.M., Cahalan M.D., Mattson M.P., and LaFerla F.M. (2000) Capacitative calcium entry deficits and elevated luminal calcium content in mutant presenilin-1 knockin mice. *J. Cell Biol.* **149,** 793–798.
109. Yoo A.S., Cheng I., Chung S., et al. (2000) Presenilin-mediated modulation of capacitative calcium entry. *Neuron* **27,** 561–572.
110. Smith D.S. and Tsai L.H. (2002) Cdk5 behind the wheel: a role in trafficking and transport? *Trends Cell. Biol.* **12,** 28–36.
111. Ohshima T., Ward J.M., Huh C.G., et al. (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proc. Natl. Acad. Sci. USA* **93,** 11,173–11,178.
112. Chae T., Kwon Y.T., Bronson R., Dikkes P., Li E., and Tsai L.-H. (1997) Mice lacking p35, a neuronal specific activator of Cdk5, display

cortical lamination defects, seizures, and adult lethality. *Neuron* **18,** 29–42.
113. Keshvara L., Magdaleno S., Benhayon D., and Curran T. (2002) Cyclin-dependent kinase 5 phosphorylates disabled 1 independently of Reelin signaling. *J. Neurosci.* **22,** 4869–4877.
114. Beffert U., Weeber E.J., Morfini G., et al. (2004) Reelin and cyclin-dependent kinase 5-dependent signals cooperate in regulating neuronal migration and synaptic transmission. *J. Neurosci.* **24,** 1897–1906.
115. Niethammer M., Smith D.S., Ayala R., et al. (2000) Nudel is a novel Cdk5 substrate that associates with Lis1 and cytoplasmic dynein. *Neuron* **28,** 697–711.
116. Tanaka T., Serneo F.F., Tseng H.C., et al. (2004) Cdk5 phosphorylation of doublecortin ser297 regulates its effect on neuronal migration. *Neuron* **41,** 215–227.
117. Assadi A.H., Zhang G., Beffert U., et al. (2003) Interaction of reelin signaling and Lis1 in brain development. *Nat. Genet.* **35,** 270–276.
118. Hocevar B.A., Mou F., Rennolds J.L., et al. (2003) Regulation of the Wnt signaling pathway by disabled-2 (Dab2). *EMBO J.* **22,** 3084–3094.
119. Yun M., Keshvara L., Park C.G., et al. (2003) Crystal structures of the Dab homology domains of mouse disabled 1 and 2. *J. Biol. Chem.* **278,** 36,572–36,581.
120. Homayouni R., Magdaleno S., Keshvara L., Rice D.S., and Curran T. (2003) Interaction of Disabled-1 and the GTPase activating protein Dab2IP in mouse brain. *Brain Res. Mol. Brain Res.* **115,** 121–129.
121. Gotthardt M., Trommsdorff M., Nevitt M.F., et al. (2000) Interactions of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. *J. Biol. Chem.* **275,** 25,616–25,624.
122. Sheen V.L., Ganesh V.S., Topcu M., et al. (2004) Mutations in ARFGEF2 implicate vesicle trafficking in neural progenitor proliferation and migration in the human cerebral cortex. *Nat. Genet.* **36,** 69–76.
123. Bajpai R., Makhijani K., Rao P.R., and Shashidhara L.S. (2004) Drosophila Twins regulates Armadillo levels in response to Wg/Wnt signal. *Development* **131,** 1007–1016.
124. Yang J., Wu J., Tan C., and Klein P.S. (2003) PP2A:B56epsilon is required for Wnt/beta-catenin signaling during embryonic development. *Development* **130,** 5569–5578.
125. Gao Z.H., Seeling J.M., Hill V., Yochum A., and Virshup D.M. (2002) Casein kinase I phosphorylates and destabilizes the beta-catenin degradation complex. *Proc. Natl. Acad. Sci. USA* **99,** 1182–1187.
126. Li X., Yost H.J., Virshup D.M., and Seeling J.M. (2001) Protein phosphatase 2A and its B56 regulatory subunit inhibit Wnt signaling in Xenopus. *EMBO J.* **20,** 4122–4131.
127. Ratcliffe M.J., Itoh K., and Sokol S.Y. (2000) A positive role for the PP2A catalytic subunit in Wnt signal transduction. *J. Biol. Chem.* **275,** 35,680–35,683.
128. Seeling J.M., Miller J.R., Gil R., Moon R.T., White R., and Virshup D.M. (1999) Regulation of beta-catenin signaling by the B56 subunit of protein phosphatase 2A. *Science* **283,** 2089–2091.
129. Sun T.Q., Lu B., Feng J.J., et al. (2001) PAR-1 is a Dishevelled-associated kinase and a positive regulator of Wnt signalling. *Nat. Cell. Biol.* **3,** 628–636.
130. Guo S. and Kemphues K.J. (1995) par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81,** 611–620.
131. Shulman J.M., Benton R., and St Johnston D. (2000) The *Drosophila* homolog of *C. elegans* PAR-1 organizes the oocyte cytoskeleton and directs oskar mRNA localization to the posterior pole. *Cell* **101,** 377–388.
132. Drewes G., Ebneth A., Preuss U., Mandelkow E.M., and Mandelkow E. (1997) MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* **89,** 297–308.
133. Bohm H., Brinkmann V., Drab M., Henske A., and Kurzchalia T.V. (1997) Mammalian homologues of *C. elegans* PAR-1 are asymmetrically localized in epithelial cells and may influence their polarity. *Curr. Biol.* **7,** 603–606.
134. Cohen D., Brennwald P.J., Rodriguez-Boulan E., and Musch A. (2004) Mammalian PAR-1 determines epithelial lumen polarity by organizing the microtubule cytoskeleton. *J. Cell Biol.* **164,** 717–727.
135. Francis F., Koulakoff A., Boucher D., et al. (1999) Doublecortin is a developmentally regulated, microtubule-associated protein expressed in

migrating and differentiating neurons. *Neuron* **23,** 247–256.
136. Gleeson J.G., Lin P.T., Flanagan L.A., and Walsh C.A. (1999) Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. *Neuron* **23,** 257–271.
137. Horesh D., Sapir T., Francis F., et al. (1999) Doublecortin, a stabilizer of microtubules. *Hum. Mol. Genet.* **8,** 1599–1610.
138. Schaar B.T., Kinoshita K., and McConnell S.K. (2004) Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons. *Neuron* **41,** 203–213.
139. Gdalyahu A., Ghosh I., Levy T., et al. (2004) DCX, a new mediator of the JNK pathway. *EMBO J.* **23,** 823–832.
140. Shmueli O., Gdalyahu A., Sorokina K., Nevo E., Avivi A., and Reiner O. (2001) DCX in PC12 cells: CREB-mediated transcription and neurite outgrowth. *Hum. Mol. Genet.* **10,** 1061–1070.
141. Bai J., Ramos R.L., Ackman J.B., Thomas A.M., Lee R.V., and LoTurco J.J. (2003) RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nat. Neurosci.* **6,** 1277–1283.
142. Tabata H. and Nakajima K. (2003) Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. *J. Neurosci.* **23,** 9996–10,001.
143. Noctor S.C., Martinez-Cerdeno V., Ivic L., and Kriegstein A.R. (2004) Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* **7,** 136–144.
144. Caspi M., Atlas R., Kantor A., Sapir T., and Reiner O. (2000) Interaction between LIS1 and doublecortin, two lissencephaly gene products. *Hum. Mol. Genet.* **9,** 2205–2213.
145. Corbo J.C., Deuel T.A., Long J.M., et al. (2002) Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. *J. Neurosci.* **22,** 7548–7557.
146. Hirotsune S., Fleck M.W., Gambello M.J., et al. (1998) Graded reduction of Pafah1b1 (Lis1) activity results in neuronal migration defects and early embryonic lethality. *Nat. Genet.* **19,** 333–339.
147. Efimov V.P. and Morris N.R. (2000) The LIS1-related NUDF protein of Aspergillus nidulans interacts with the coiled-coil domain of the NUDE/RO11 protein. *J. Cell Biol.* **150,** 681–688.
148. Hoffmann B., Zuo W., Liu A., and Morris N.R. (2001) The LIS1 related protein NUDF of *Aspergillus nidulans* and its interaction partner NUDE bind directly to specific subunits of dynein and dynactin and to {alpha}- and {gamma}-tubulin. *J. Biol. Chem.* **16,** 16.
149. Sasaki S., Shionoya A., Ishida M., et al. (2000) A LIS1/NUDEL/cytoplasmic dynein heavy chain complex in the developing and adult nervous system. *Neuron* **28,** 681–696.
150. Feng Y., Olson E.C., Stukenberg P.T., Flanagan L.A., Kirschner M.W., and Walsh C.A. (2000) LIS1 regulates CNS lamination by interacting with mNudE, a central component of the centrosome. *Neuron* **28,** 665–679.
151. Sweeney K.J., Prokscha A., and Eichele G. (2001) NudE-L, a novel Lis1-interacting protein, belongs to a family of vertebrate coiled-coil proteins. *Mech. Dev.* **101,** 21–33.
152. Kitagawa M., Umezu M., Aoki J., Koizumi H., Arai H., and Inoue K. (2000) Direct association of LIS1, the lissencephaly gene product, with a mammalian homologue of a fungal nuclear distribution protein, rNUDE. *FEBS Lett.* **479,** 57–62.
153. Fanto M. and McNeill H. (2004) Planar polarity from flies to vertebrates. *J. Cell Sci.* **117,** 527–533.
154. Weston C.R. and Davis R.J. (2002) The JNK signal transduction pathway. *Curr. Opin. Genet. Dev.* **12,** 14–21.
155. Miller J.R., Rowning B.A., Larabell C.A., Yang-Snyder J.A., Bates R.L., and Moon R.T. (1999) Establishment of the dorsal–ventral axis in *Xenopus* embryos coincides with the dorsal enrichment of dishevelled that is dependent on cortical rotation. *J. Cell Biol.* **146,** 427–437.
156. Winter C.G., Wang B., Ballew A., et al. (2001) *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. *Cell* **105,** 81–91.
157. Fanto M., Weber U., Strutt D.I., and Mlodzik M. (2000) Nuclear signaling by Rac and Rho GTPases is required in the establishment of epithelial planar polarity in the Drosophila eye. *Curr. Biol.* **10,** 979–988.
158. Pazman C., Mayes C.A., Fanto M., Haynes S.R., and Mlodzik M. (2000) Rasputin, the *Drosophila* homologue of the RasGAP SH3 binding protein, functions in ras- and Rho-mediated signaling. *Development* **127,** 1715–1725.

159. Strutt D.I., Weber U., and Mlodzik M. (1997) The role of RhoA in tissue polarity and Frizzled signalling. *Nature* **387,** 292–295.
160. Habas R., Kato Y., and He X. (2001) Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1. *Cell* **107,** 843–854.
161. Wasserman S. (1998) FH proteins as cytoskeletal organizers. *Trends Cell Biol.* **8,** 111–115.
162. Carreira-Barbosa F., Concha M.L., Takeuchi M., Ueno N., Wilson S.W., and Tada M. (2003) Prickle 1 regulates cell movements during gastrulation and neuronal migration in zebrafish. *Development* **130,** 4037–4046.
163. Morrison D.K. and Davis R.J. (2003) Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu. Rev. Cell. Dev. Biol.* **19,** 91–118.
164. Whitmarsh A.J., Cavanagh J., Tournier C., Yasuda J., and Davis R.J. (1998) A mammalian scaffold complex that selectively mediates MAP kinase activation. *Science* **281,** 1671–1674.
165. Yasuda J., Whitmarsh A.J., Cavanagh J., Sharma M., and Davis R.J. (1999) The JIP group of mitogen-activated protein kinase scaffold proteins. *Mol. Cell. Biol.* **19,** 7245–7254.
166. Willoughby E.A., Perkins G.R., Collins M.K., and Whitmarsh A.J. (2003) The JIP-1 scaffold protein targets MKP-7 to dephosphorylate JNK. *J. Biol. Chem.* **10,** 10.
167. Verhey K.J., Meyer D., Deehan R., et al. (2001) Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. *J. Cell Biol.* **152,** 959–970.
168. Bowman A.B., Kamal A., Ritchings B.W., et al. (2000) Kinesin-dependent axonal transport is mediated by the sunday driver (SYD) protein. *Cell* **103,** 583–594.
169. Byrd D.T., Kawasaki M., Walcoff M., Hisamoto N., Matsumoto K., and Jin Y. (2001) UNC-16, a JNK-signaling scaffold protein, regulates vesicle transport in C. elegans. *Neuron* **32,** 787–800.
170. Reiner O., Gdalyahu A., Ghosh I., et al. (2004) DCX's phosphorylation by not Just aNother Kinase (JNK). *Cell Cycle* **3,** Website, http://www.landesbioscience.com/ 747–751.
171. Bonny C., Nicod P., and Waeber G. (1998) IB1, a JIP-1-related nuclear protein present in insulin-secreting cells. *J. Biol. Chem.* **273,** 1843–1846.
172. Dickens M., Rogers J.S., Cavanagh J., et al. (1997) A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* **277,** 693–696.
173. Negri S., Oberson A., Steinmann M., et al. (2000) cDNA cloning and mapping of a novel islet-brain/JNK-interacting protein. *Genomics* **64,** 324–330.
174. Meyer D., Liu A., and Margolis B. (1999) Interaction of c-Jun amino-terminal kinase interacting protein-1 with p190 rhoGEF and its localization in differentiated neurons. *J. Biol. Chem.* **274,** 35,113–35,118.
175. Matsuda S., Yasukawa T., Homma Y., et al. (2001) c-Jun N-terminal kinase (JNK)-interacting protein-1b/islet-brain-1 scaffolds Alzheimer's amyloid precursor protein with JNK. *J. Neurosci.* **21,** 6597–6607.
176. Scheinfeld M.H., Roncarati R., Vito P., Lopez P.A., Abdallah M., and D'Adamio L. (2002) Jun NH2-terminal kinase (JNK) interacting protein 1 (JIP1) binds the cytoplasmic domain of the Alzheimer's beta-amyloid precursor protein (APP). *J. Biol. Chem.* **277,** 3767–3775.
177. Taru H., Kirino Y., and Suzuki T. (2002) Differential roles of JIP scaffold proteins in the modulation of amyloid precursor protein metabolism. *J. Biol. Chem.* **277,** 27,567–27,574.
178. Stockinger W., Brandes C., Fasching D., et al. (2000) The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2. *J. Biol. Chem.* **275,** 25,625–25,632.
179. Buchsbaum R.J., Connolly B.A., and Feig L.A. (2002) Interaction of Rac exchange factors Tiam1 and Ras-GRF1 with a scaffold for the p38 mitogen-activated protein kinase cascade. *Mol. Cell. Biol.* **22,** 4073–4085.
180. Pleasure S.J. (2001) An arrow hits the Wnt signaling pathway. *Trends Neurosci.* **24,** 69–71.
181. Hall A.C., Lucas F.R., and Salinas P.C. (2000) Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell* **100,** 525–535.
182. Wang Y., Huso D., Cahill H., Ryugo D., and Nathans J. (2001) Progressive cerebellar, auditory, and esophageal dysfunction caused by targeted disruption of the frizzled-4 gene. *J. Neurosci.* **21,** 4761–4771.
183. Ridley M. (ed.) (1987) *The Darwin Reader.* WW Norton, New York.
184. Kirschner M. and Gerhart J. (1998) Evolvability. *Proc. Natl. Acad. Sci. USA* **95,** 8420–8427.