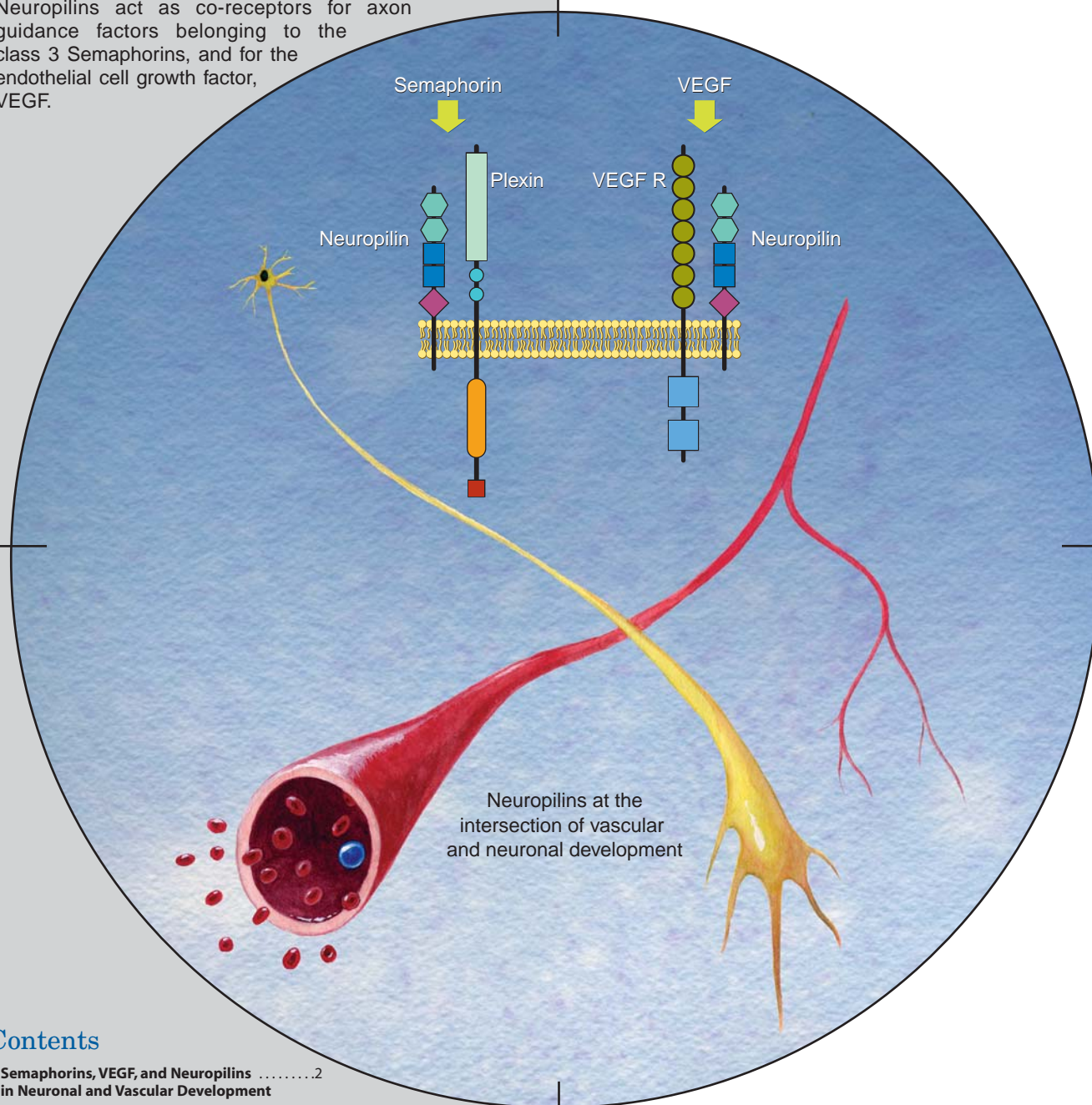


The nervous and vascular systems exhibit striking parallels in terms of anatomy and development, both forming complex branching networks. It has become evident that parallels exist at the molecular level as well. Neuropilins act as co-receptors for axon guidance factors belonging to the class 3 Semaphorins, and for the endothelial cell growth factor, VEGF.



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R&D Systems offers an array of proteins for the study of cell survival, cell differentiation, and axon guidance.

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- CNTF
- EGF
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- NT-3
- NT-4
- PDGFs
- VEGFs

Morphogens

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- Hedgehogs
- Wnt-3a

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- Netrins
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- NCAMs
- Laminin I

Please visit www.RnDSystems.com/TrophicGuidance for a complete product listing.

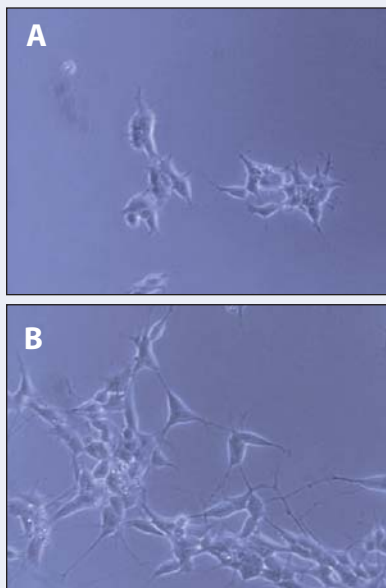


Figure 1. Human neuroblastoma cells (CRL-2266) grown under control conditions (A) are less differentiated than those grown in the presence of R&D Systems' recombinant mouse Artemin protein (B) (Catalog # 1085-AR; 20 ng/mL).

Semaphorins, VEGF, and Neuropilins in Neuronal and Vascular Development

The nervous and vascular systems exhibit striking parallels in terms of anatomy and development. Both nerves and vessels form complex branching networks throughout the body.¹ In addition, both of these cell types form and retract filopodia to precisely guide their paths through areas of attractive and repulsive forces.^{2,3} Nerves and vessels often follow similar routes and means of migration during embryogenesis.¹ One of the most prominent examples occurs in the vertebrate limb, where major nerves of the peripheral nervous system are co-localized with major arteries. These neurovascular bundles supply muscles and bones, and this phenomenon has been termed neurovascular congruence.⁴ A hypothesis to explain neurovascular congruency suggests that the nervous and vascular systems use shared patterning mechanisms involving such signaling molecules as semaphorins, vascular endothelial growth factor (VEGF), and neuropilins. In fact, ectopic expression of Semaphorin 3A (Sema3A) in developing chick forelimbs causes altered pathways of peripheral nerves and hypovascularization, and suggests that the role of Sema3A is to refine neurovascular patterning.⁴ Neurovascular congruence in the developing limb serves as an example of the striking interplay of a small number signaling factors involved in both nervous system and cardiovascular development.

Semaphorins are key mediators of axon guidance, acting by inhibiting axonal motility, causing repulsive effects, and collapsing growth cones.⁵ Semaphorins are also implicated in other processes during neuronal development such as axonal fasciculation, neuronal migration, dendritic guidance, and in the remodeling and repairing of the adult nervous system.⁵ In addition, Sema3A impacts the development of the vasculature: it inhibits endothelial cell motility, prevents microvessel outgrowth and sprouting, and disrupts lamellipodia formation and cytoskeletal organization.⁶ These effects are dependent on Sema3A binding Neuropilin-1 (Npn-1) expressed on the surface of endothelial cells, indicating that Sema3A functionally interacts with Npn-1 in non-neural cells.⁶

VEGF induces blood vessel growth and influences cell motility and survival of endothelial cells.¹ It also plays important roles in the nervous system such as increasing axon outgrowth, promoting invasion of Schwann cells, and encouraging neovascularization, key events during nerve regeneration.^{7,8} Furthermore, VEGF promotes survival of motor neurons⁹ and stimulates neurogenesis in the adult mammalian brain via multiple intracellular pathways.¹⁰

Neuropilins are membrane proteins that function as receptors for the Class-3 Semaphorin subfamily as well as for certain splice forms of VEGF (see cover).¹¹ Substitution of just 7 amino acids in Npn-1 completely disrupts Semaphorin, but not VEGF binding.¹² Utilization of this conditional Npn-1 mutation in mice (Npn-1^{Sema}) has enabled more precise dissection of Npn function *in vivo*. Sema/Npn-1 signaling is necessary for axonal pathfinding by cranial and spinal nerves, for promoting fasciculation of some peripheral nerves as well as for guiding peripheral projections and several populations of CNS neurons.¹² Sema/Npn-1 signaling is not required for general vascular development, as there were no discernible vascular defects, but it is required for certain aspects of heart development.¹² While general vascular development appeared normal in these Npn-1^{Sema} mice, other studies indicate that endogenous Sema3A in endothelial cells is important for vascular development. Sema3A is necessary for suppressing integrin function, and for allowing de-adhesion between endothelial cells and the extracellular matrix, essential roles in the morphogenetic process of angiogenic remodeling.¹³

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Transcription Factors and Early Nervous System Development

Understanding the mechanisms underlying neural fate and the development of specific neuronal subtypes are fundamental issues in the field of developmental neuroscience. Secreted inductive signals stimulate the regional activation of transcription factors associated with key steps in nervous system development including the formation of neural plate from ectodermal tissue, neural tube formation, and finally differentiation into specific neuronal subtypes.

In the early *Xenopus* embryo, bone morphogenetic protein (BMP)-4, a TGF- β superfamily member, induces ectodermal tissues to adopt an epidermal fate, while inhibiting the "default" formation of neural plate tissues.¹ Several proteins that promote the formation of neural tissues via binding and inhibition of BMPs have been identified and include Noggin, Follistatin, Chordin, Cerberus, and *Xenopus* Nodal-related 3 (Xnr3).²⁻⁶ Transcription factors including Msx1 and Dlx3 lie downstream of BMPs and mediate anti-neural effects,⁷ while important factors involved in the induction of the neural phenotype include Smad-7 and Neurogenin (Xngnr in *Xenopus*).^{8,9} The activities of Smad-7 and Neurogenin that occur in the absence of BMP signals result in the activation of transcription factors including Ath-3 and NeuroD, basic helix-loop-helix (bHLH) proteins that participate in later steps of primary neural differentiation.¹⁰⁻¹²

The process by which the neural plate forms a neural tube is called neurulation. Neurulation requires the expression of Pax3, a paired box gene expressed exclusively during embryogenesis.¹³ Pax3 regulates neural tube morphogenesis by inhibiting p53-dependent apoptosis.¹⁴ The early neural tube is composed of germinal neuroepithelium made up of a layer of rapidly dividing neural stem cells. The differentiation of the neural tube into the various regions of CNS is controlled initially by BMP-4 and BMP-7 from the epidermis, and Sonic Hedgehog (Shh) from the notochord. Secondary regulation is then established within the neural tube by BMP-4 secreted from roof plate and Shh secreted from the floor plate. Graded ventral to dorsal Shh signals establish distinct progenitor domains along the dorsal/ventral axis of the neural tube that give rise to different classes of postmitotic neurons.¹⁵⁻¹⁸ The boundaries of the progenitor domains are delineated according to the Shh-dependent expression of distinct combinations of transcription factors.¹⁹⁻²¹ For example, the expression of Pax6, Irx3, Dbx2, Dbx1, Pax3, and Pax7 is repressed by Shh signals (Class I), whereas Shh stimulates the expression of Nkx2.2, Nkx2.9, Olig2, Nkx6.1, and Nkx6.2 (Class II). Domains are further refined through reciprocal inhibition by opposing class I and class II factors (Figure 1).

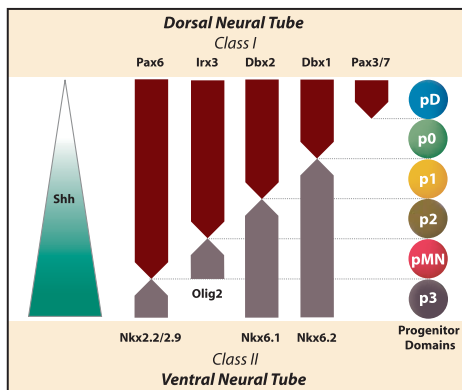


Figure 1. A ventral to dorsal concentration gradient of Shh induces class II transcription factors while repressing class I transcription factors. Opposing class I and class II factors are reciprocally inhibitory leading to the formation of distinct progenitor domains (pD, p0, p1, p2, pMN, and p3) that give rise to unique cell types. [Note: figure adapted from Lee, S.-K. & S.L. Pfaff (2001) *Nat. Neurosci. Suppl.* 4:1183.]

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Stem Cell Research Kits & Reagents

Developmental neurobiology begins with the study of stem and progenitor cells and their differentiation into neural lineage-committed cells. R&D Systems offers neural stem cell differentiation and expansion kits as well as antibodies specific for a variety of stem, progenitor, and neural cell lineage markers.

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- SSEA-3
- SSEA-4
- Nestin

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- NCAM-L1
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- Noggin
- O1
- O4
- Pax6
- Tyrosine Hydroxylase

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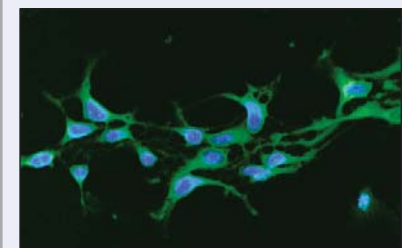


Figure 1. Detection of Nestin in human week-11 fetal neural progenitor cells using R&D Systems' mouse anti-human Nestin monoclonal antibody (Catalog # MAB1259). Cells were stained using anti-mouse Alexa Fluor[®] 488 (green) and counterstained with DAPI (blue).

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	Protein	Antibody	ELISA/ Assays	Primer Pair		Protein	Antibody	ELISA/ Assays	Primer Pair
Activin A	◆◆◆	◆◆◆			EphB4	●	●		
Activin AB	◆				EphB6	●	●		
Activin B	◆	◆			Ephrin-A1	●	●		
Activin C		●			Ephrin-A2	●	●		
Activin RIA/ALK-2	◆	◆			Ephrin-A3	◆	◆		
Activin RIB/ALK-4	◆	◆●			Ephrin-A4	◆●	◆●		
Activin RIIA	◆	◆			Ephrin-A5	◆	◆		
Activin RIIB	◆	◆			Ephrin-B1	●	●		
Agrin	■	■			Ephrin-B2	●★	●★		
ALK-1	◆●	◆●			Ephrin-B3	◆	◆		
ALK-7	■	■	■		ErbB2	◆	◆		
Artemin	●	●			ErbB3	◆	◆	◆	
BDNF	◆	◆	◆		ErbB4	◆	◆		
BMP-2	◆★	◆	◆		FGF acidic	◆□	◆□	◆	
BMP-3	◆	◆			FGF basic	◆□	◆□	◆	◆
BMP-4	◆★	◆★	◆		FGF-BP	■	◆■		
BMP-5	◆	◆			FGF R1	◆	◆		
BMP-6	◆	◆	◆		FGF R2	◆●	◆●		
BMP-7	◆	◆	◆		FGF R3	◆●	◆●	◆	
BMP-8		◆			FGF R4	◆	◆●		
BMPR-IA/ALK-3	◆●	◆			FGF-3	◆	◆		
BMPR-IB/ALK-6	◆●	◆●			FGF-4	◆	◆	◆	
BMPR-II	◆	◆			FGF-5	◆	◆		
Chordin	●	●			FGF-6	◆	◆	◆	
CNTF	◆■	◆■	◆■		FGF-8	●	◆●		
CNTF Rα	◆■	◆■			FGF-9	◆	◆	◆	
Collagen I	■□				FGF-10/KGF-2	◆	◆		
Collagen IV	●				FGF-16	◆			
Cripto/Cripto-1	◆●	◆			FGF-17	◆	◆		
Cryptic	◆				FGF-18	◆	◆		
DAN	◆●	◆●			FGF-19	◆	◆		
DCC	●	●			Fibronectin	□			
Desert Hedgehog		●			Frizzled-1		◆●		
Dkk-1	◆	◆			Frizzled-2	●	●		
Dkk-3		◆●			Frizzled-3		◆●		
Dkk-4	◆	◆			Frizzled-4	●	●		
DLL4	◆●				Frizzled-6		●		
EGF	◆	◆	◆		Frizzled-7	◆	◆●		
EGF R	◆	◆●	◆		Frizzled-8	●	●		
EphA1	◆	◆			sFRP-1	◆	◆		
EphA2	●				sFRP-2		●		
EphA3	●	●			sFRP-3	◆●	◆●		
EphA4	●	●			GDF-1		●		
EphA5	■	■			GDF-3		●		
EphA6	●	●			GDF-5	●	●		
EphA7	●	●			GDF-6	●			
EphA8	●	●			GDF-7	●			
EphB1	■	■			GDF-8	●	●		
EphB2	●	●			GDF-9		●		
EphB3	●	●			GDF-15/MIC-1	◆	◆		

Key

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● Mouse

■ Rat

★ Porcine

○ Amphibian

□ Bovine

✦ Chicken

▼ Multi-species

★ Zebrafish

These products are for research use only. Not for use in humans or diagnostic procedures.

	Protein	Antibody	ELISA/ Assays	Primer Pair		Protein	Antibody	ELISA/ Assays	Primer Pair
GDNF	◆	◆	◆						
GFAP				◆ ●					
GFRα-1	◆	◆							
GFRα-2	◆	◆							
GFRα-3	◆	◆							
GFRα-4		◆							
gp130	◆	◆	◆						
Gremlin	●	●							
Growth Hormone	◆	◆							
Growth Hormone R	◆	◆							
Hip	●	●							
IGF-I	◆	◆	◆						
IGF-I R	◆	◆	◆						
IGF-II	◆	◆	●						
IGFBP-1	◆	◆	◆						
IGFBP-2	◆	◆							
IGFBP-3	◆	◆	◆						
IGFBP-4	◆	◆	◆						
IGFBP-5	◆	◆	●						
IGFBP-6	◆	◆	◆						
IGFBP-7		◆							
Insulin		◆							
Insulin R	◆								
Integrin α2/CD49b		◆							
Integrin α3/CD49c		◆							
Integrin α4/CD49d		◆							
Integrin α6/CD49f		◆							
Integrin αv/CD51		◆							
Jagged 1	◆	◆							
KGF/FGF-7	◆	◆	◆						
Laminin I	●								
MAG/Siglec-4a	◆	◆							
NCAM		◆		◆					
NCAM-L1	◆	◆							
Neogenin	●	●							
Nestin		◆		◆					
Netrin-1	●	●							
Netrin-2	●	●							
Netrin-4	◆	◆							
Netrin-G1a	●								
Neuregulin-1-α/HRG-α	◆	◆	◆						
Neuregulin-1-β1/HRG-β1	◆	◆	◆						
Neuregulin-3		●							
Neuropilin-1	◆	◆							
Neuropilin-2	◆	◆		◆					
Neurotrimin		●							
Neurturin	◆	◆							
β-NGF	◆	◆	◆	◆					
NGF R/TNFRSF16	◆	◆		◆					
Nodal	●	●							
Noggin	●	●							
Nogo R	◆	◆							
Nope	●								
Notch-1	◆								
Notch-2	◆	◆							
Notch-3	◆	●							
NT-3	◆	◆	◆						
NT-4	◆	◆	◆						
O1		◆							
O4		◆							
OCAM/NCAM-2		●							
PDGF-AA	◆	◆	◆						
PDGF-AB	◆		◆						
PDGF-BB	◆	◆	◆						
PDGF-C	◆	◆							
PDGF-D	◆	◆							
PDGF Rα	◆	◆		◆					
PDGF Rβ	◆	◆		◆					
Ret	◆	◆							
Semaphorin 3A	◆								
Semaphorin 6A	◆	◆							
Sonic Hedgehog	◆	◆	●						
SOST	◆	◆							
LAP (TGF-β1)	◆	◆							
Latent TGF-β1	◆		◆						
Latent TGF-β1 BP1 (LTBP-1)		◆							
TGF-β1	◆	◆	◆	◆					
TGF-β1.2	◆								
TGF-β2	◆	◆	◆	◆					
TGF-β3	◆	◆							
TGF-β5	○	◆							
TGF-β RI/ALK-5	●	●		◆					
TGF-β RII	◆	◆							
TGF-β RIIb	◆								
TGF-β RIII	◆	◆							
TrkA	◆	◆							
TrkB	◆	◆							
TrkC	◆	◆							
TSG	●	●							
UNC5H1	◆								
UNC5H2	◆	◆							
UNC5H3	◆	◆							
UNC5H4	◆								
Wnt-3a	●								
Wnt-4		●							
Wnt-5a		●							
VEGF	◆	◆	◆	◆					
VEGF R1/Flt-1	◆	◆	◆	◆					
VEGF R2/KDR	◆	◆	◆	◆					

Key

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ADAM10	◆ ●	◆ ●
APP		◆ <i>Pan Specific</i>
BACE-1	◆	◆
Cathepsin D	◆ ●	◆ ●
Cathepsin E	◆ ●	◆
Cystatin B	◆ ●	
Cystatin C	◆ ●	
Nephrilysin	◆ ●	◆ ●
Parkin-1		◆
Presenilin-1		◆
Presenilin-2		◆
Synuclein- α		◆
TACE/ADAM17	◆	◆
N-Me-6,7-diOH-TIQ		◆ ●
Ubiquitin	◆	◆

Key: ◆ Human ● Mouse

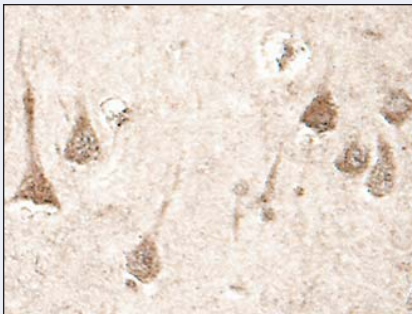


Figure 1. Detection of Presenilin-1 N-terminal fragment (NTF) in paraffin-embedded human hippocampal tissue sections using R&D Systems' goat anti-human Presenilin-1 NTF polyclonal antibody (Catalog # AF149). Tissues were stained using R&D Systems' Goat HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS008; brown) and counter-stained with hematoxylin (blue).

R&D Systems also offers α -, β -, and γ -Secretase Activity Assays for monitoring the activities of the enzymes responsible for the formation of amyloid beta peptide, a major component of Alzheimer's disease plaques.

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Desert Hedgehog, Schwann Cells, and Peripheral Nerve Development

Standard histology texts describe the architecture of the mature peripheral nerve as composed of three layers; a centrally placed endoneurium, a thin, laterally placed perineurium, and a poorly-defined external, connective tissue-rich epineurium. As would be anticipated, this architecture reflects developmental processes involved in its formation. Until recently, the nature and sequence of these events has gone unappreciated. Research with both rat and mouse models has now provided considerable insight into mammalian nerve development, with an invaluable contribution made by the Desert Hedgehog knockout mouse.

Peripheral nerve development begins with the formation of the neural crest, an aggregation of specialized ectodermal cells adjacent to the neural tube. These cells can assume a variety of phenotypes under the influence of various mediators.¹⁻³ In mouse on day E12, possibly under the influence of Neuregulin-1- β 1, Schwann cell (SC) precursors emerge from the neural crest and accompany outgrowing axons from the region of the neural tube.^{1,4} This is a reciprocal relationship that provides a continuum of support cells for migrating axons, and axonally-produced Neuregulin-1- β 1 trophic support for migrating SC precursors. By day E14 in mouse, precursor SCs begin developing into immature SCs.^{1,5,6} Postnatally, under the influence of molecules like FGF basic, immature SCs mature first into myelinating SCs that cover motor axons, and later into nonmyelinating SCs that line small diameter axons carrying autonomic and sensory impulses.^{1,2,5}

Depending upon their location, SCs can support a number of activities. If they are immature and near motor neurons, they can produce GDNF, a trophic factor for neurons.⁵ If they are mature and lining an axon, they can secrete autocrine survival factors such as PDGF-BB, NT-3 and IGF-II.^{1,2} At day E11.5, SCs begin to express a secreted molecule known as Desert Hedgehog (Dhh).⁶⁻⁸ Dhh is considered to have a membrane or local effect, and binds to the Patched (Ptc) receptor appearing on mesenchymal cells on mouse day E15.⁹ Binding to Ptc induces a mesenchymal-to-epithelial cell transformation with the ultimate formation of the perineurial sheath.⁴ Initially, and independent of Dhh, mesenchyme is drawn towards the axon-SC complex where a thin, permeable sheet is formed. The mesenchyme is transformed into epithelium via Dhh activity.⁴ The newly transformed epithelial cells become flat, fuse laterally via tight junctions, and form a pavement-like structure that encircles multiple axon-SC complexes. The structures encased within a perineurial sheath are collectively called a fascicle. In a typical mammalian perineurial sheath, there are up to 15 layers of perineurial lamellar sheets.¹⁰ This multilayer perineurium encloses multiple axon-SC complexes plus the surrounding endoneurium, which is composed of collagen, fibroblasts, and mast cells.^{9,10} The squamous, or pavement-like nature of the perineurium provides a barrier to unwanted cell and molecule intrusion into the axon-SC area.^{1,4,10} Dhh knockout mice exhibit abnormalities in the connective tissue sheaths in peripheral nerves (Table 1). For instance, the definitive perineurium is significantly thinner, and small groups of perineurial cells are ectopically placed within the endoneurium forming minifascicles. In mice, this may negatively impact axon conduction velocity.⁴ Thus, it would appear that Dhh plays a central role in the development of peripheral nerve integrity.

Table 1. Peripheral nerve phenotype of the Desert Hedgehog knockout mouse.

- ✓ Epineurium is virtually absent
- ✓ Perineurium is abnormally thin
- ✓ Ectopic perineurial cells present in endoneurium form mini fascicles
- ✓ Perineurial cells fail to express connexin 43
- ✓ Compromised diffusion and infiltration barrier
- ✓ Lower motor nerve conduction velocity

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CREB and the Molecular Neurobiology of Memory

Memory formation is believed to proceed through three general stages.¹ The initial stage, acquisition, involves the perception of a new experience. The middle stage, short-term memory (STM), is defined as a transient formation of this newly acquired experience. The final stage, long-term memory (LTM), consolidates STMs into durable recollections, and unlike STM requires mRNA and protein synthesis. One signal transduction cascade, the Raf/MEK/ERK pathway, has garnered considerable attention for its LTM role in both developing and mature animals.² Much of the attention has converged on a phosphorylated target of this pathway, the cAMP response element binding protein (CREB).

CREB belongs to the bZIP superfamily of transcription factors, containing a basic domain that mediates DNA binding and a leucine zipper domain that facilitates dimerization.³ Within the promoter of target genes, CREB dimers bind cAMP response elements (CREs), defined by the palindromic consensus sequence TGACGTCA. When phosphorylated at Ser133, CREB also binds the coactivator CREB binding protein (CBP), which enhances transcription by acetylating histones to facilitate chromatin unraveling.⁴ The transcription cascade regulated by CREB is proposed to initiate synapse-specific structural changes in a remarkable cellular network of 100 billion neurons with 7 trillion connections among them.¹ These changes in synapse structure, termed synaptic plasticity, are the physical basis of LTM.

Experiments with *Aplysia*, *Drosophila*, and more recently with rodents, support a vital role for CREB in memory formation. In mice, robust CREB phosphorylation and CRE-reporter gene expression are detected in neurons during developmental plasticity and memory training.⁵ During contextual fear conditioning, CREB null mice display deficits in LTM, but not STM.⁶ Similar deficits are detected in spatial learning using the Morris water maze and mice injected with CREB antisense.⁷ In experiments pairing lemon odor with foot shock, CREB phosphorylation is required for aversive olfactory learning in young rats.⁸ Consistent with these findings, CREB is needed for proper nervous system development. In knockouts lacking both

CREB and the related transcription factor CREM, a massive loss of neurons is detected in mouse cortex, striatum, and hippocampus, a critical compartment for STM to LTM processing.⁹

Three naturally occurring human genetic mutations that result in mental retardation and other learning disorders have shed additional light on CREB's role in memory and cognition, and have also implicated the ERK pathway that activates CREB (Figure 1). First, neurofibromatosis is caused by disruptive mutations in the GAP-encoding neurofibromatosis type 1 oncogene (NF1).¹⁰ Inactive NF1 fails to down-regulate the GTP-binding protein Ras, leading to inappropriate activation of Raf, MEK, ERK and all direct and indirect downstream ERK targets. Further downstream, disruption of the gene encoding RSK2 causes a second form of mental retardation, the Coffin-Lowry Syndrome.¹¹ ERK signaling couples to CREB through intervening kinases of the RSK family, which phosphorylate and activate CREB at the critical Ser133. A third form of mental retardation associated with CREB is the Rubinstein-Taybi Syndrome (RTS).¹² The RTS gene encodes CBP, and offending mutations eliminate the coactivator's histone acetyltransferase activity, diminishing the transcription of plasticity-associated genes.

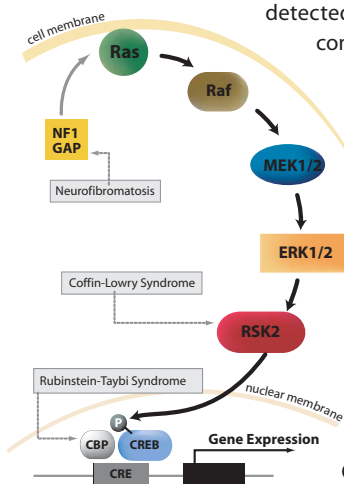


Figure 1. The Ras/Raf/ERK signaling cascade leading to CREB phosphorylation and gene expression has been implicated in the development of learning and memory. Three naturally occurring mutations that affect this pathway result in learning and memory deficits and retardation. [Note: figure adapted from Weeber, E. & J. Sweatt (2002) *Neuron* **33**:845.]

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Phospho-ERK1/ERK2 DuoSet® IC

As part of the Raf/MEK/ERK signal transduction module, the Ser/Thr kinases ERK1 and ERK2 regulate cellular proliferation, differentiation, and survival. Both ERKs are activated by dual phosphorylation, at T202/Y204 for human ERK1 and T185/Y187 for human ERK2.¹ In the nucleus, active ERKs phosphorylate a number of transcription factors, including TCFs² and Myc.³ ERK1 and ERK2 also regulate transcription indirectly by phosphorylating kinases from the RSK⁴ and MSK⁵ families.

The phospho-ERK1/ERK2 DuoSet IC ELISA kit (Catalog # DYC1018) joins R&D Systems' Signal Transduction Kits & Reagents group of products for measurement of phosphorylated intracellular signaling factors. DuoSet IC (Intracellular) ELISA Development Systems provide a fast, economical, and quantitative alternative to Western blots that can be easily adapted to high throughput analysis. The utility of the phospho-ERK1/ERK2 DuoSet IC is demonstrated in Figure 1.

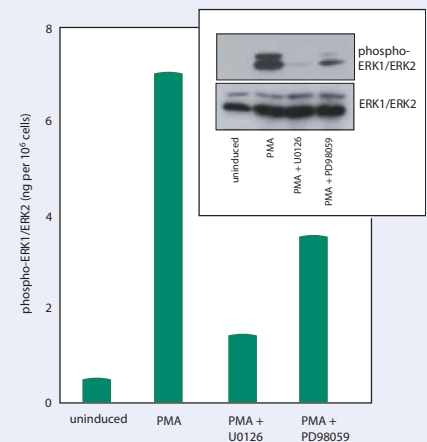


Figure 1. HeLa cells were incubated for 20 minutes with or without 200 nM PMA and with or without MEK inhibitors U0126 or PD98059. Cells were lysed and phosphorylated ERK1 and ERK2 were quantified using R&D Systems' phospho-ERK1/ERK2 DuoSet IC ELISA kit (Catalog # DYC1018). The same lysates were also immunoblotted (inset) with either anti-phospho-ERK1/ERK2 (Catalog # AF1018) or anti-ERK1/ERK2 (Catalog # MAB1576) antibodies. The DuoSet IC results correlate well with the amounts of phosphorylated ERK1 and ERK2 detected by Western blot.

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Wnt-3a

in Nervous System Development and Pathogenesis

Wnts make up a family of small, secreted, glycoproteins that play critical roles in nervous system development ranging from the regulation of neuronal and neural crest fate, to axon guidance and synaptogenesis.¹⁻⁴ One Wnt family member specifically, Wnt-3a, is expressed in the developing mouse nervous system, often in complementary patterns with its close relative, Wnt-3. Wnt-3a is found in the roof plate of the developing spinal cord and in the midline of the brain, with expression extending into the bifurcating telencephalon.⁵ *In vitro*, Wnt-3a increases the number of pigment cells in neural crest cell cultures, at the expense of neuronal and glial cells, and Wnt-3a/Wnt-1-deficient mice exhibit a reduction in the neural crest cell population.^{6,7} Forebrain explant assays suggest that Wnt-3a has the ability to regulate transcription factors responsible for the development of anterior/posterior patterning.⁸ Wnt-3a null mice exhibit homeotic transformation of their vertebrae, abnormal somites, a disrupted notochord, and CNS dysmorphology including the absence of all hippocampal structures.^{9,10} Recent evidence suggests that Wnt-3a may also play a role in regulating the pathogenesis of Alzheimer's disease (AD). Neuronal A β toxicity is accompanied by a downregulation of Wnt activity and toxicity is blocked by treatment with Wnt-3a, indicating a potential for future AD therapies.¹¹

In murine pre-osteoblast MC3T3-E1 cells, R&D Systems' recombinant Wnt-3a (Catalog # 1324-WN; Figure 1) synergizes with recombinant BMP-2 (100 ng/mL; Catalog # 355-BM) to induce alkaline phosphatase (AP). Treatment with increasing Wnt-3a concentrations results in the formation of a bell-shaped dose-response curve (Figure 2). For details regarding the AP induction assay, please visit our website at: <http://www.RnDSystems.com/Wnt-3a>

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Figure 1. Silver-stained 12% SDS polyacrylamide gel showing R&D Systems' recombinant mouse Wnt-3a protein (Catalog # 1324-WN) under reducing conditions.

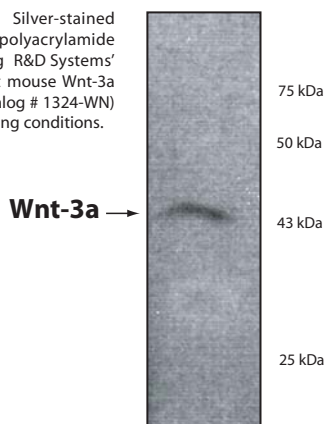
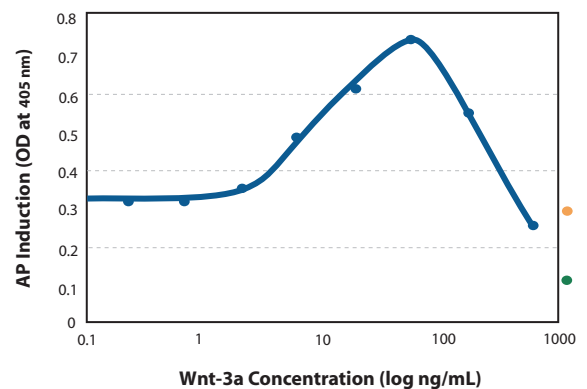


Figure 2. In murine pre-osteoblast MC3T3-E1 cells, R&D Systems' recombinant Wnt-3a (Catalog # 1324-WN) synergizes with recombinant BMP-2 (100 ng/mL; Catalog # 355-BM) to induce alkaline phosphatase (AP; blue). At higher Wnt-3a concentrations, AP induction declines resulting in a bell-shaped dose-response curve. AP induction by BMP-2 only (100 ng/mL; orange), and untreated controls (green) is shown at the right side of the graph.



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