<table>
<thead>
<tr>
<th>Allele</th>
<th>turtle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation Type</td>
<td>missense</td>
</tr>
<tr>
<td>Chromosome</td>
<td>11</td>
</tr>
<tr>
<td>Coordinate</td>
<td>69,557,283 bp (GRCm38)</td>
</tr>
<tr>
<td>Base Change (assembly)</td>
<td>A ? G</td>
</tr>
<tr>
<td>Gene</td>
<td>Efnb3</td>
</tr>
<tr>
<td>Gene Name</td>
<td>ephrin B3</td>
</tr>
<tr>
<td>Synonym(s)</td>
<td>Epi8, EFL-6, Elk-L3, ELF-3, LERK-8, NLERK-2</td>
</tr>
<tr>
<td>Chromosomal Location</td>
<td>69,554,092-69,560,205 bp (-)</td>
</tr>
<tr>
<td>Accession Number</td>
<td>Ncbi Refseq: NM_007911.5; MGI: 109196</td>
</tr>
<tr>
<td>Mapped</td>
<td>Yes</td>
</tr>
<tr>
<td>Amino Acid Change</td>
<td>Leucine changed to Proline</td>
</tr>
<tr>
<td>Institutional Source</td>
<td>Beutler Lab</td>
</tr>
<tr>
<td>Phenotypic Category</td>
<td>behavior/neurological</td>
</tr>
<tr>
<td>Penetration</td>
<td></td>
</tr>
<tr>
<td>Alleles Listed at MGI</td>
<td>All alleles(9) : Targeted(9)</td>
</tr>
<tr>
<td>Mode of Inheritance</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>Local Stock</td>
<td>Sperm</td>
</tr>
<tr>
<td>Repository</td>
<td>MMRRC:36909</td>
</tr>
<tr>
<td>Science Writers</td>
<td>Tiana Purrington, Bruce Beutler</td>
</tr>
<tr>
<td>Illustrators</td>
<td>Diantha La Vine</td>
</tr>
<tr>
<td>Last Updated</td>
<td>05/22/2018 9:45 AM by Anne Murray</td>
</tr>
<tr>
<td>Record Created</td>
<td>03/14/2012 10:14 AM by Bruce Beutler</td>
</tr>
<tr>
<td>Record Posted</td>
<td>03/28/2014</td>
</tr>
</tbody>
</table>

Cite this information as follows: Tiana Purrington, Anne Murray, Beutler B. Record for turtle, updated May 22, 2018. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu
The *turtle* mouse was visually identified among G3 mice homozygous for mutations induced by N-ethyl-N-nitrosourea (ENU) by a defect in their ability to right themselves when placed on their back and an inability to stay standing (*Figure 1*; *turtle* displays normal behavior (e.g., feeding and grooming). Older animals exhibit a “hopping” or mirror movements when walking and running (*Figure 2*).
Whole genome SOLiD sequencing of the G1 grandsire identified 19 mutations. Three G3 mice with the turtle phenotype and six unaffected mice were genotyped at all 19 mutation sites and three mutations on chromosome 11 were homozygous in all three of the turtle mice but none of the unaffected mice. Capillary sequencing of the mutated genes identified a T to C transition at base pair 69370785 (v37) on Chromosome 11 in the GenBank genomic region NC_000077 encoding Efnb3 (Figure 3). The mutation corresponds to residue 332 in the mRNA sequence within exon 2 of 5 total exons.

The mutated nucleotide is indicated in red. The mutation results in a leucine (L) to proline (P) substitution at residue 111.

**Protein Prediction**

Efnb3 encodes the highly conserved (i.e., homology between human and mouse ephrin-B3 is >96%) ephrin-B3, a member of the membrane-associated ephrin family of ligands (1-3). The ephrin ligands and the ephrin (Eph) tyrosine kinase receptors each have two subfamilies, ephrin-A and ephrin-B or EphA and EphB, respectively (1;3). Ephrin-A ligands preferentially bind to EphA receptors (caveat: the EphA4 receptor (see the record for frog) can also recognize ephrin-B3), while ephrin-B ligands bind preferentially to EphB receptors (1;4-7). Ephrin-B3, in particular, binds with nanomolar affinity to EphB3, but does not have high affinity for the other EphB receptors (i.e., EphB1, EphB2, or EphB4) (1).

The ephrin-B ligand subfamily has three members: ephrin-B1 (alternatively LERK-2/Elf-L/Cek5-L), ephrin-B2 (alternatively, ELF-2/Htk-L), and ephrin-B3; ephrin-B3 shares 48.8% and 49.3% amino acid homology with ephrin-B1 and ephrin-B2, respectively (1). Ephrin-B ligands are anchored to the membrane by a hydrophobic transmembrane domain [amino acids 228-248 in ephrin-B3; Figure 4; (1;3)]. The N-terminus of ephrin-B3 (amino acids 1-27) is a signal peptide for secretion (1), amino acids 28-227 are extracellular, and a highly conserved cytoplasmic tail (amino acids 249-340) can become tyrosine phosphorylated as well as associate with SH2 and PDZ domain-containing proteins (3;4;8;9).

Cite this information as follows: Tiana Purrington, Anne Murray, Beutler B. Record for turtle, updated May 22, 2018. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu
that the heparin-binding region is within amino acids 178-194 [(10); see “Background”, below, for more details]. Site-directed mutagenesis of four basic residues within this region (Arginine (Arg; R)178, Lysine (Lys; K)179, Arg188, and Arg190) determined that, upon substitution of a glycine (Gly; G) for Arg178 or an asparagine (Asn; N) for Lys179, heparin binding was decreased; a double mutant (R178G/K179N) did not bind heparin at all (10).

Substitutions of Arg188 or Arg190 for glycines caused no change in binding (10). Taken together, these results indicate that Arg178 and Lys179 constitute a binding site for heparin (10). Arg188 was identified as an essential amino acid for binding of ephrin-B3 to B lymphocytes [(11); see “Background”, below, for more details]. Reelin also associates with the extracellular domain of ephrin-b3 [(12); see “Background”, below, for more details]. The cytoplasmic domain of ephrin-B3 is required to mediate reverse signaling events (see “Background” for more details) as well as to interact with SH2/SH3 adaptor molecules (e.g., Grb4; alternatively, Nck2), tyrosine phosphatases (e.g., PTP-BL; alternatively, Ptpn13), and PDZ adaptor molecules (e.g., GRIP1/2 and PDZ-RGS3) [(13;14); reviewed in (15)]. Examination of ephrin-B3 cytoplasmic deletion mutants determined that the regulation of synaptic numbers, regulation of the expression of synaptosomal protein GRIP, and the phosphorylation of PKA are all dependent on the cytoplasmic domain (13;14). Although ephrin-B3 contributes to regulating synaptic transmission, modulation of synaptic protein expression, and hippocampal-dependent memory, these processes are independent of its cytoplasmic domain (13). Upon axon-dendrite contact and postsynaptic ephrin-B3 association with a presynaptic EphB receptor, ephrin-B3 can recruit Grb4 or Pick1/syntenin via SH2 or PDZ binding motifs, respectively (3). Grb4 and Pick1 bridge ephrin-B3 to cytoskeletal regulators, receptors and ion channels thereby altering their subcellular targeting and/or surface expression to subsequently facilitate bi-directional signaling (3;8;16-18). For example, Grb4 can couple ephrin-B3 to Dock180 and PAK, leading to signaling downstream of Rac and Cdc42 to function in dendritic growth and remodeling as well as in mossy fiber axon pruning (18;19).

The transmembrane domains of B-type ephrins are targets for cleavage by human RHBDL2, an intramembrane rhomboid protein (see the record for sinecur for more information about rhomboid proteases); human rhomboids RHBDL1 and RHBDL4 did not cleave ephrin-B3 (2). In vitro analysis of an ephrin-B3 truncated at Arg214, Pro226, or Cys250 determined that the Pro226 truncation mutant was similar in molecular weight to the protein product cleaved by RHBDL2, indicating that ephrin-B3 is cleaved close to this residue (2). The physiological relevance of RHBDL2-mediated cleavage of ephrin-B3 remains to be elucidated (2). However, Pascall et al. propose that release of a soluble form of ephrin-B3 functions as an inhibitor of Eph/Ephrin-mediated cell interactions (2).

Figure 5. Crystal structure of the ephrin-B2 ectodomain. The ectodomain is an eight-stranded β-barrel arranged in two sheets (the mixed parallel and antiparallel β-strands are arranged in a Greek key topology) around a hydrophobic core; two β-helices and one 3₁₀ helix is interspersed between the β-strands. Key residues of interest are labeled in the color that coordinates to the location in the crystal structure; functionally significant residues are described in the text. Area I (cyan), Area II (red), and residues common to both (green) are shown as well as the disulfide bonds between Cys92-Cys156 (peach) and Cys65-Cys104 (purple). The figure is based on PDB: 1IKO.

Cite this information as follows: Tiana Purrington, Anne Murray, Beutler B. Record for turtle, updated May 22, 2018. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu

(Generated on Jun 12, 2018)
The crystal structure of the ephrin-B2 ectodomain has been solved and associates as dimers [PDB: 1IKO; (7); Figure 5]. The ectodomain is an eight-stranded alpha barrel arranged in two sheets (the mixed parallel and antiparallel strands are arranged in a Greek key topology) around a hydrophobic core; two alpha helices and one 3_10 helix are interspersed between the strands (7). Two buried disulfide bonds increase the stability of the ectodomain: the bond between Cys65 and Cys104 connects strands C and F; Cys92 and Cys156 bond anchors two small helices, E and J, at the top of the barrel (7). Similar to other ephrins, ephrin-B2 is N-linked glycosylated at Asn39, but the function of this posttranslational modification is unknown (7).

The ephrin-B3 ligand can bind the EphB receptors, EphA4, heparin, an unknown heparan-sulfated proteoglycan (HSPG) receptor on HeLa and HEK-293T cells, and an unidentified protein expressed on B cells (11;18;20). Ephrin-B3 has a putative heparin/heparin sulfate-binding domain that is unique from its Eph-receptor-binding domain (10). Signaling through the HSPG receptor regulates cell morphology and cell spreading; ephrin-B3 binding to the receptor can be blocked by heparin (10). B-lymphocytes (namely CD19+ B lymphocytes) are the only peripheral blood mononuclear cells that can also bind ephrin-B3 (11). The receptor on the B cell is unknown; Holen et al. propose that it is not an EphB receptor or a HSPG (11). Stimulation by ephrin-B3 leads to increased migration of IgD+ memory B lymphocytes; naïve B lymphocytes did not migrate as efficiently as the memory subpopulation (11). Ephrin-B3 also enhances Cxcl12- and Ccl19-induced migration of B lymphocytes (11). Ephrin-B3 binding to B cells is downregulated following B cell activation, pointing to a possible role for ephrin-B3-B cell association in non-stimulated cells (11).

The *turtle* mutation is a leucine to proline substitution at residue 111 within the extracellular ephrin receptor binding domain (RBD; aa 28-167; UniProt).
In situ hybridization of whole mount mouse embryos detected strongest Efnb3 expression in the floor plate of the midbrain and hindbrain; weaker expression was detected in the dorsal hindbrain, in rhombomeres 2,4,6 \(^{(1)}\). At embryonic day (E) 14, Efnb3 was strongly expressed in the preoptic area and along the superficial migratory stream \(^{(21)}\). At E15 and 16, the expression was detected in the dorsal midline of the hindbrain and spinal cord \(^{(1;22)}\). At postnatal day (P) 0, Efnb3 expression was detected in the mouse midbrain in mesostriatal dopaminergic neurons \(^{(20)}\). At P10.5, 11.5, and 12.5 expression was detected in the floor plate \(^{(1;23;24)}\). In situ hybridization has also detected high levels of Efnb3 in the granule layer of the hippocampal dentate gyrus and in CA1 neurons \(^{(25;26)}\).

Ephrin-B3 can be either pre- \(^{(13;27)}\) or postsynaptic \(^{(25;28)}\). Antibody labeling of ephrin-B3 detected strong expression of ephrin-B3 in floor plate cells of the developing nervous system \(^{(24)}\). In primary dissociated rat cortical neurons, at 5-10 days \(in vitro\), ephrin-B3 is enriched at excitatory synapses and also localized to many extrasynaptic locations \(^{(28)}\). At 14-21 days \(in vitro\), dendritic spine formation occurs and ephrin-B3 is largely restricted to spine and shaft excitatory synaptic contacts \(^{(28)}\). In postnatal tissues, ephrin-B3 is expressed in the myelinating oligodendrocytes in the mouse spinal cord, and optic nerve as well as in tissues surrounding the subventricular zone and rostral migratory stream of the brain (e.g., cortex, striatum, septum, and corpus callosum); expression of ephrin-B3 is not detected at the midline in adults or within the subventricular zone and rostral migratory stream \(^{(29-33)}\). Additional studies detected high levels of the ephrin-B3 protein within the dentate gyrus in the hilus and the inner molecular layer; lower levels were detected in the outer molecular layer \(^{(5)}\). The \(Efnb3^{-/-}\) mouse has defective cell positioning of stem/progenitor cells outside of the subgranular zone of the hippocampal dentate gyrus as well as abnormal cellular processes extending into the hilus \(^{(5)}\). Ephrin-B3 is also localized to post-synaptic CA1 dendrites and pre-synaptic mossy fibers \(^{(3;13;18)}\). Although ephrin-B3 expression has been detected in the adult brain, it is not a phosphorylated form, indicating that it may not be active in physiological processes of the adult brain \(^{(4)}\). Ephrin-B3 is also expressed in the white pulp of the spleen: 30.4% of resting T cells and 30.1% of activated (24 hours) T cells express ephrin-B3 \(^{(34)}\). Expression of ephrin-B3 on CD4 and CD8 T cells was similar (26.1 and 22.1%, respectively); the CD4 cells expressed more of the ephrin-B3 receptor than CD8 cells (45.4 and 21.3%, respectively) \(^{(34)}\). The expression of ephrin-B3 or its receptor was barely detectable in B cells, but a significant percentage of monocytes/macrophages expressed ephrin-B3 (20.4%) \(^{(34)}\). In the cancer cell lines U251 and SNB19, ephrin-B3 was localized to the leading edge at sites of lamellipodial formation \(^{(4)}\).

Background
Figure 7. Overview of ephrin-B-mediated bidirectional signaling. In forward signaling, the Eph receptor is autophosphorylated upon receptor-ligand interaction on several juxtamembrane tyrosine residues. GRB4 (growth factor receptor bound protein-4) subsequently interacts with the receptor to facilitate signaling that results in changes in integrin activity and reorganization of the actin cytoskeleton. In reverse signaling, upon interaction with the Eph receptor, the ephrin ligand is phosphorylated, resulting in the association of GRB4 to the PDZ domain. In ephrin-B1-mediated signaling, GRB4 can subsequently recruit signaling molecules (e.g., FAK, Axin, Abi1, CAP, and Cbl [not shown]) to the signaling complex, eventually leading to changes in actin dynamics (see the record for frog for more information on EphA4/ephrin-B-mediated signaling affects on actin dynamics). Ephrin-B signaling can also induce the downregulation of MAPK/ERK pathway. Ephrin-B3 induces the phosphorylation of the NR2 subunit of the NMDA receptor (NMDAR), which subsequently functions in long-term potentiation (LTP), learning, memory, synaptic maturation, and synaptogenesis. Ephrin-B3 can also function in Reelin signaling. The secreted extracellular protein, Reelin, binds to ephrin-B proteins and subsequently associates at the membrane with the Reelin receptors, VLDLR and ApoER2 (not shown). Reelin-associated signaling functions in regulating axonal transport, neuronal migration, microtubule dynamics, and actin reorganization during neuronal migration in the brain.

Ephrin-B3-mediated signaling can regulate several functions including apoptosis (40), T-cell signaling (34), axon guidance to regulate corticospinal tract projection (47), and axon pruning (11;18;20).

Ephrin-B3 and the central nervous system

Regulation of NMDA receptors

Regulation of the NMDA receptors modulates glutamatergic synaptic transmission, a process essential for the development and function of the central nervous system (48). Ephrin-B3 hypomorphic mice have an enhanced ratio of NMDA receptor- to AMPA receptor-mediated excitatory postsynaptic currents (EPSCs) (48). Hypomorphic mice have reduced excitatory postsynaptic potential amplitudes and a reduction in synaptic weight, indicating that they have fewer AMPA receptors; synaptic NMDA receptors were not altered (48). Ephrin-B3 limits EphB2-mediated phosphorylation of the N-Methyl-D-Aspartate (NMDA) receptor NR2 subunit; phosphorylation of the subunit is enhanced in Ephb3 mice (25;48). EphB2 interaction with the NMDA receptors in the postsynaptic density (PSD) of excitatory synapses in CA1 neurons of the hippocampus modulates calcium influx into the postsynaptic cells to subsequently function in long-term potentiation (LTP), learning, memory, synaptic maturation, and synaptogenesis (13;25;41;48;49). Taken together, these studies show that ephrin-B3 is required for the regulation of the NMDA receptor and maturation of glutamatergic synaptic transmission (48). In support of this, Ephb3 mice have reduced CA3-CA1 LTP along with defective learning and memory tasks (13).
signaling \((28;50)\). In vitro, shRNA-mediated knockdown of Efnb3 leads to a significant reduction in dendritic spine density, spine synapses, and shaft synapses \((28;43)\). In Efnb3\(^{-/-}\) mice, the density of dendritic protrusions was significantly reduced with a concomitant reduction in dendritic spine density; synapse density was not changed \((28)\). McClelland et al. addressed the apparent discrepancy between the in vitro knockdown and in vivo knockout data \((28)\). They determined that the relative level of ephrin-B3 can selectively increase or decrease the density of synapses, suggesting that cell-to-cell differences in ephrin-B3 expression enables neuronal competition to control synapse density \((28)\). To support this, heterogenotypic cultures of Efnb3\(^{-/-}\) and wild-type neurons showed that neurons expressing higher levels of ephrin-B3 formed more synapses and that there was competition for the presynaptic ligand, EphB2 \((28)\). Expression of a dominant negative MEK rescued any defects in the heterogenotypic cultures, indicating that ephrin-B3-mediated control of synapse density is dependent on ERK signaling \((28)\). Furthermore, McClelland et al. determined that ephrin-B3 interacts with ERK and subsequently negatively regulates ERK signaling \((28)\).

**Ephrin-B3 and Reelin signaling**

During the development of laminated structures and neuronal migration in the brain, ephrin-B proteins (i.e., ephrin-B1, ephrin-B2 and ephrin-B3) facilitate Reelin signaling, a signaling pathway essential for central nervous system (CNS) development \((12)\). Compound mouse mutants (null for ephrin-B3 or ephrin-B2 and heterozygous for Reelin) and a triple ephrin-B1, B2, B3 knockout exhibited neuronal migration defects with a significant increase in neurons in the marginal zone as well as defects in hippocampal laminated structures \((12)\). Reelin, an extracellular protein secreted by neurons, binds to the extracellular domain of ephrin-B proteins and subsequently associates at the membrane with Reelin receptors, VLDLR and ApoER2 \((12)\). Binding of Reelin to ephrin-B3 also leads to ephrin-B3 clustering, a process necessary for the recruitment and phosphorylation of Dab1, an adaptor protein necessary for Reelin signaling \((12)\).
Figure 8. Ephrin-B3 serves as a midline repellant during corticospinal tract (CST) pathfinding. (top) Model of embryonic brain anatomy. Color divisions match the coronal sections shown below. (bottom) Coronal sections through the forebrain (top, pink), hindbrain (middle, purple), and spinal cord (bottom, green) are shown. In the views of the CST, the left side cortical neurons are colored in red, the right side neurons are colored in blue. The spinal cord section shows the CST fibers located as a fasciculated longitudinal bundle in the contralateral dorsal column. Collateral fibers branch off the midline fascicle and project into the ventral horn to innervate the appropriate contralateral motor neurons, which are colored according to their cortical input. Ephrin-B3 is concentrated at the midline of the nervous system and prevents collateral fibers of the CST from projecting across the midline. Figure is adapted from Yokoyama et al. (2001).

Ephrin-B3 is a midline repellant.
capsule/pyramidal tract (51) (Figure 8). In the caudal medulla, the growth cones of the corticospinal tract cross the midline and travel down the spinal cord in the contralateral dorsal column, forming a bundle (51). Ephrin-B proteins function in promotion of the collapse of commissural growth cones at this stage, helping to prevent aberrant crossing of the midline (24). At the appropriate site of the spinal cord, individual axons branch and enter the contralateral side of the spinal cord to innervate the appropriate motor neurons (51). The axons only cross the midline once and are prevented from crossing back over through ephrin-B3-induced EphA4 signaling (29;47;51;52). Disruption of the EphA4 (or EphB) and ephrin-B3 interaction cause decussated (i.e., crossed or intersected) commissural axons to inappropriately invade the dorsal regions of the spinal cord (24).

Axon growth, guidance and regeneration
In addition to its role as a midline repellent, ephrin-B3 is also proposed to function in regulating the growth and guidance of axons in the injured adult CNS (20;21;24;30;36;51). Duffy et al. found that after CNS trauma (e.g., optic nerve crush or dorsal hemisection of the thoracic spinal cord), ephrin-B3 in myelinating oligodendrocytes, along with the myelin-derived inhibitors Nogo, MAG, and OMgp, limits recovery through its inhibition of axon growth (30). In Ephb3 knockout animals (Ephb3<sup>-/-</sup>), corticospinal and raphespinal growth in the caudal spinal cord after CNS injury was significantly improved over wild-type animals (30). Furthermore, the Ephb3<sup>-/-</sup> animals had improved locomotor function (30). After optic nerve crush injury, the Ephb3<sup>-/-</sup> mouse also had significantly greater optic nerve regeneration (30). In vitro, CNS myelin extracts inhibited neurite outgrowth of dorsal root ganglion cells; loss of ephrin-B3 expression reduced this inhibitory activity (30). Another study found that ephrin-B3 supports adult retinal ganglion cell axon growth in vitro (36). In Ephb3<sup>-/-</sup> mice, loss of ephrin-B3 expression led to a decrease retinal ganglion cell axon sprouting after optic nerve injury (36). This study found that the loss of axonal plasticity was not due to a change in the growth potential of the axon after injury (36). A third study found that after transient focal cerebral ischemia, neurogenesis is increased in the Ephb3<sup>-/-</sup> mouse without a concomitant neurological recovery (38). However, signal transducer and activator of transcription 1 (STAT1)-associated brain injury is enhanced (38) Doepfner et al. determined that the Ephb3<sup>-/-</sup> mouse had increased cell proliferation rates of neural progenitor cells as well as increased astroglial and neuronal differentiation rates (38).

Neurogenesis and apoptosis
The subventricular zone (SVZ) is a region of the brain in which neurogenesis occurs in adults (53). Neural progenitor cells in the SVZ differentiate into neuroblasts that terminally differentiate into interneurons in the olfactory bulb (32;54;55). In the adult brain, blockade of ephrin-B3 results in increased proliferation and migration of SVZ-derived neural precursor cells (32;33). In Ephb3<sup>-/-</sup> mice, cell proliferation was increased along the lateral ventricle; the increased proliferation in these animals could be reduced by the infusion of soluble ephrin-B3 (32). Ricard et al. propose that ephrin-B3-mediated signaling (via EphB3 or EphA4 receptors) upregulates cell cycle activators (e.g., PCNA) and downregulates cell cycle inhibitors (e.g., cyclin-dependent kinase inhibitor p27Kip1) (32). In addition to its role in cell proliferation, ephrin-B3 can also regulate cell apoptosis in neurogenic regions in the adult (32). In adult mice, loss of ephrin-B3 expression leads to an increase in TUNEL-positive cells in the SVZ (32;40). Ephrin-B3 can prevent EphA4-mediated pro-apoptotic effects: EphA4 is a dependence receptor that is cleared by caspase-3-like caspase, leading to caspase-dependent cell death in the absence of ephrin-B3 (40). In an ephrin-B2 study, Sawamiphak et al. determined that ephrin-B2 functions in cell apoptosis by regulating the activity of vascular endothelial growth factor (VEGF) receptor 2 (56); the role of ephrin-B3 in the regulation of VEGFR2 activity has not been examined.

Ephrin-B3 and cancer
Studies have shown that ephrin-B3 functions in cancer progression. Ephrin-B3 is upregulated in migrating glioma cell lines and changes in the expression of ephrin-B3 alters the invasion and migration of human glioma cells: siRNA-mediated knockdown inhibits invasion and migration, while forced expression induces invasion and migration both in vitro and ex vivo (4). In addition, the phosphorylation level of ephrin-B3 directly correlated with the amount of glioma migration and invasion rates (4). Further examination determined that ephrin-B3 mediates the changes in glioma invasion by activating Rac1, a protein known to stimulate cell migration through actin reorganization and the formation of lamellipodia (4;57;58). In U-810 cells, a non-small cell lung cancer cell line, ephrin-B3 silencing led to decreased proliferation and a change to a flattened, elongated morphology (from a small round morphology) of the cells (44). To determine the ephrin-B3 signaling network that could be contributing to these changes in the U-810 cells, ephrin-B3-dependent changes in the phosphorylation status of the proteome were examined by mass spectrometry (44). Stahl et al. determined a network of proteins, including active EphA2 and focal adhesion kinase 1 (FAK1), are involved in ephrin-B3-mediated cell survival signaling to mediate changes in growth, morphology and invasiveness (44). Stahl et al. propose that ephrin-B3 blocks the interaction of ephrin-A1 with the EphA2 receptor, Akt1 subsequently phosphorylates EphA2, activating downstream signaling through FAK1 (44). In general, the proteins in the network function to control cellular assembly and organization, cellular development, and cell morphology (44). This study confirmed that ephrin-B3 binds to and results in phosphorylation of the EphA2 receptor, subsequently preventing the binding of ephrin-A1 to the receptor and promoting cell survival (44).

Ephrin-B3 and T cell signaling
Ephrin-B3 has a role in T-cell activation and function. In vitro stimulation of T cells by ephrin-B3 in the presence of anti-CD3 resulted in increased proliferation and cytotoxic T lymphocyte activity, production of IFN-γ, and the expression of certain T-cell activation markers (e.g., CD69) (34). T cell receptor (TCR) crosslinking using anti-CD3 antibodies resulted in congregation of ephrin-B3 receptors (various Ephs) with TCRI's in aggregated lipid rafts, providing a physical basis for the potential interaction between TCR and Eph pathways (34). Yu et al. propose that ephrin-B3 is important for T-cell/T-cell and T-cell/antigen-presenting cell collaboration to encourage T-cell activation and function (34).

Ephrin-B3 as a hepinavirus receptor

Cite this information as follows: Tiana Purrington, Anne Murray, Beutler B. Record for turtle, updated May 22, 2018. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu

(Generated on Jun 12, 2018)
Nipah virus in pigs and Hendra virus in horses); human infection occurs after contact with either the infected pigs or horses (59-62). Henipavirus entry in the cell is through the attachment of the viral G envelope glycoprotein with either of the ephrin-B proteins, followed by activation of the F fusion protein (a type-I transmembrane glycoprotein), and subsequent fusion of the viral envelope and the host membrane (62). Ephrin-B3 interacts with the viral G envelope glycoprotein at the hydrophobic region on the G-H loop, which includes residues Trp125, Leu124, Pro122, and Tyr/Phe120 [PDB: 3D12; (60;62;63)]; the leucine and tryptophan residues are critical for binding of the Nipah virus G protein (60). Upon interaction with the G protein, the ephrin-B3 G-H loop undergoes a conformational change to enhance binding of ephrin-B3 to the G protein (62). Interaction of ephrin-B3 with the henipavirus is not proposed to induce ephrin-B3-mediated reverse signaling (62).

Putative Mechanism

The Efnb3−/− mouse exhibits a kangaroo/rabbit-like “hopping” phenotype similar to turtle [MGI: 3026700; (51) and MGI: 2388046; (47;52)]. Yokoyama et al. observed that the corticospinal tract of the Efnb3−/− mouse was defective in that axons bilaterally innervated both contralateral and ipsilateral motor neuron populations, indicating that ephrin-B3 functions as a midline repellant (51). Further studies by Kullander et al. demonstrated that the “hopping” gate of the Efnb3−/− mouse was linked to defects in local spinal neuronal networks that generate and coordinate the contraction and relaxation of muscles, termed central pattern generators (52). This study determined that the normal rhythmic pattern in isolated spinal cords from Efnb3−/− mouse was defective at 0 to 5 days, and therefore independent of the corticospinal tract (52). Bilateral ventral roots exhibited an abnormal synchronous rhythm as opposed to the wild-type left-right alternation (52). Ipsilateral lumbar (L) segment 2 and L5 ventral roots had a normal alternating pattern in the knockouts (52). In another study, Restrepo et al. found that in EphA4 knockout mice that there was an increase in the number of crossing excitatory neurons and an increase in inhibitory neurons crossing the midline compared to EphA4 heterozygous mice (64). Taken together, these findings indicate that ephrin-B3-induced EphA4 signaling (see the record for frog) functions to repel components of the spinal central pattern generators and restricts their axonal projections to one side of the spinal cord (52). In addition, due to changes to EphA4 signaling in the Efnb3−/− mouse, the balance between excitatory and inhibitory signals across the midline are altered leading to the hopping phenotype (52;64).

The phenotype observed in turtle indicates that the leucine to proline mutation at residue 111 is detrimental to ephrin-B3-mediated signaling. It is possible that this mutation impairs the ability of ephrin-B3 to bind to the EphA4 or EphB3 receptor, thus rendering it unable to function in bidirectional signalling during axon guidance.
Turtle genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change. The following primers are used for PCR amplification:

**Primers for PCR amplification**

Turtle(F): 5’-CGACTGAATGCCAAGGGAGCATTAC -3’
Turtle(R): 5’-GGGAGCTAAGAATTGCTGTCCGAG -3’

**Primers for sequencing**

Turtle_seq(F): 5’-CTGAATACCCAGTGGTGGATTCC -3’

**PCR program**

1) 94°C 2:00
2) 94°C 0:30
3) 57°C 0:30
4) 72°C 1:00
5) repeat steps (2-4) 29x
6) 72°C 7:00
7) 4°C

The following sequence of 515 nucleotides is amplified (Chr. 11: 69557082-69557596, GRCm38; NC_000077):

```
cgactgaatgccaagggagtattacctgaatccaccagttggattccaatggctctaacc

ttcctccccctccatccccacggacactctatttgtgttgatctcgacgtctggcagtacca

ttgaaactgtagtggtggtggtggcggactgtgcctgtgcgctggctcagcagcagctcgaccattctggtgcgggagtggccggggtggtggtggcgcctccagctttccctccctcctcccctcgttggttggtggtggcgcctccagctttccctccctcctcctgcctggacagctccgaaccagaaagttaaggtgtgcgcgcacaaagcaggcagggaggtgtcagggtgtggccgcctcccaacca
```

Primer binding sites are underlined; sequencing primers are highlighted; the mutated nucleotide is highlighted in red (A>G, Chr. + strand; T>C, sense strand).

**References**

Mutagenetix Phenotypic Mutation 'turtle'

Mutagenetix Phenotypic Mutation 'turtle'


Cite this information as follows: Tiana Purrington, Anne Murray, Beutler B. Record for turtle, updated May 22, 2018. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu

(Generated on Jun 12, 2018)