Megalin and the neurodevelopmental biology of sonic hedgehog and retinol

Robert A. McCarthy and W. Scott Argraves*

Medical University of South Carolina, Department of Cell Biology, 171 Ashley Avenue, Charleston, SC 29425-2204, USA
*Author for correspondence (e-mail: argraves@musc.edu)

Journal of Cell Science 116, 955-960 © 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00313

Summary

Megalin is a receptor expressed by embryonic epithelia that mediates endocytosis of numerous ligands, including sonic hedgehog (Shh) and retinol, the precursor to retinoic acid (RA). The importance of Shh and RA signaling in neurodevelopment, combined with the fact that megalin-deficient mice show profound neurodevelopmental abnormalities, has raised questions as to the possible role of megalin in Shh and RA signaling. Several mechanisms could explain how megalin influences Shh and RA signaling in the context of neurodevelopment. These include the involvement of megalin in the transport of Shh and retinol within neuroepithelia, as well as direct signal transduction as a response to binding of Shh and retinol to megalin.

Key words: Hedgehog, Retinol, Retinoic acid, Endocytosis, Transcytosis, Holoprosencephaly

Introduction

Megalin (gp330/LRP-2) is a member of the low-density lipoprotein (LDL) receptor family (Fig. 1). It is expressed exclusively in epithelial cells of the embryo and the adult, in which it mediates the endocytosis of an array of ligands (Barth and Argraves, 2001). Considering the diversity of ligands that can interact with megalin, it is not surprising that mice lacking megalin display a range of embryonic abnormalities and that most die perinataly (Willnow et al., 1996). Studies of megalin$^{-/-}$ survivors have provided significant insights into the role of megalin in adult physiology. For example, renal megalin is critical for vitamin homeostasis in adults, mediating proximal tubule uptake of complexes of vitamins and their carriers, including steroid 25-(OH) vitamin D3 bound to vitamin D binding protein, vitamin A (retinol) bound to retinol-binding protein and vitamin B_{12} bound to transcobalamin (Christensen and Willnow, 1999). In megalin-deficient mice these vitamins are excreted in the urine. The resulting deficiency in vitamin D leads to bone disease (Nykjaer et al., 1999). In contrast to its role in adult physiology, we know little about how megalin so profoundly influences embryonic development. Megalin-deficient embryos display numerous craniofacial abnormalities, including an absence of olfactory bulbs, absence of the corpus callosum and fusion of forebrain hemispheres hallmarks of holoprosencephaly (HPE) (Willnow et al., 1996) (Table 1). The HPE phenotype indicates that megalin is essential for the formation of the nervous system. Here we review recent findings that provide new insights into the possible role(s) of megalin in neurodevelopment.

Megalin expression in development

Megalin is expressed on trophectoderm cells in the developing mouse embryo as early as the blastocyst stage (Buc-Caron et al., 1987). Later in development (8 days post coitum, dpc), it is highly expressed by cells of the extraembryonic visceral endoderm of the yolk sac and several other polarized epithelia, including the amnion, cardiac endothelium and the neural tube (Buc-Caron et al., 1987). As development progresses, megalin expression persists on apical surfaces of neural epithelia, including the neural canal, choroid plexus and ependymal cells lining the ventricles (Kounnas et al., 1994; Willnow et al., 1996). Additionally, megalin is prominently expressed by epithelial cells of the developing ear, thyroid, lung, renal proximal tubules and intestine (Kounnas et al., 1994). In all cases megalin expression is limited to the apical surfaces of the plasma membrane.

Megalin and regulation of retinol-dependent development

Megalin binds to and endocytoses complexes of retinal-binding protein and retinol (Christensen et al., 1999). Furthermore, this is critical for transepithelial transport of retinol by renal-proximal tubule epithelial cells (Christensen et al., 1999; Marino et al., 2001). Given these findings and the well established importance of retinol in early development (Means and Gudas, 1995), it is possible that megalin is a regulator of retinol metabolism critical for embryonic development. There are at least two possible mechanisms in which megalin might play a role. Firstly, megalin might directly mediate endocytosis of retinol by cells that synthesize retinoic acid (RA). Secondly, megalin may mediate transepithelial transport of retinol and thereby facilitate delivery of retinol to cells that synthesize RA.

With respect to the first possibility, megalin would be expected to be expressed by cells that also express retinaldehyde dehydrogenases (Raldh), enzymes that oxidize retinal to RA. However, there is evidence that this is not the case. In the mouse, there are three *Raldh* genes, *Raldh1-Raldh3*. The pattern of cells in the developing mouse embryo that express Raldh1-3 (Mic et al., 2002) does not closely overlap with that of megalin expression (Kounnas et al., 1994).

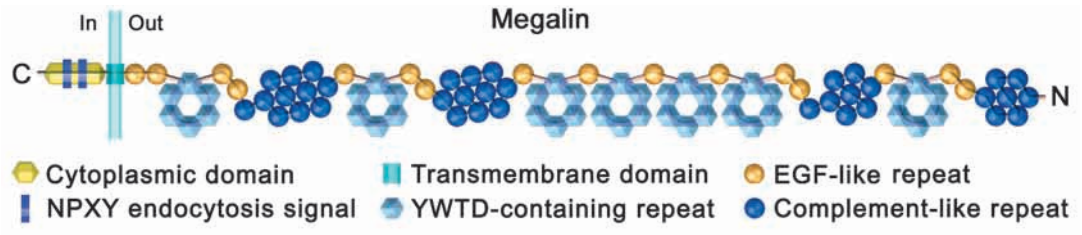

Fig. 1. Schematic diagram depicting the modular structure of megalin. Megalin (LRP-2) is a large, ~600 kDa integral membrane protein belonging to the LDL receptor gene family [for a review of the LDL receptor gene family see (Nykjaer and Willnow, 2002; Strickland et al., 2002)]. Its cytoplasmic domain contains PxxP, NPxY, YxxM and LL elements that are thought to be involved in the endocytosis and signaling activities of the receptor. Numerous adaptor molecules have been shown to interact with the megalin cytoplasmic domain including proteins with PTB, PDZ domains as well as ones with ankyrin and tetratrico repeat elements (Gotthardt et al., 2000; Petersen et al., 2003; Rader et al., 2000). The ectodomain of megalin comprises several distinct domains or sequence motifs including ligand-binding-type cysteine-rich repeats (complement-like), epidermal growth factor type cysteine-rich repeats and YWTD domains [each group of six YWTD repeats folds into a beta-propeller structure (Springer, 1998)].

Raldh1 is expressed in the ectoderm of the dorsal retina of the eye (E10.5) (Mic et al., 2002) and the substantia nigra of the developing brain (E15.5) (Smith et al., 2001). Raldh2 is highly expressed in the mesoderm of the trunk region of murine embryos (E8-E9) but absent in the developing brain (Smith et al., 2001). Smith et al. have proposed that RA produced by this trunk mesoderm diffuses both dorsally and medially to developing hindbrain tissue (Smith et al., 2001). Raldh3 is expressed in surface ectoderm of the optic vesicle (9-10.5 dpc) (Mic et al., 2002; Smith et al., 2001). In contrast to these patterns of Raldh gene expression, megalin is initially expressed in the floor plate and along apical margins of the neural tube (McCarthy et al., 2002) and later in the choroid plexus and ependymal layer of the brain (Kounnas et al., 1994). The fact that megalin and the known *Raldh* genes do not seem to be expressed in the same tissues argues against megalin playing a role as a mediator of retinol uptake by cells that synthesize RA. However, a recent study in which Raldh2-deficient embryos were conditionally rescued shows sites of RA synthesis that do not correspond to sites of expression of the known *Raldh* genes (Mic et al., 2002) but show some overlap with sites of megalin expression. For example, novel sites of RA synthesis include the floorplate and neural tube, which are known to also express megalin (McCarthy et al., 2002). Therefore, the possibility that megalin mediates uptake of retinol by a subset of RA-synthesizing cells, particularly neural epithelial cells, must still be considered.

If megalin plays a role in transport of retinol to cells that synthesize RA, there are two possibilities: one, megalin expressed by extraembryonic visceral endoderm (VE) mediates transport of maternally derived retinol to the embryo; and two, megalin expressed on apical surfaces of certain

Table 1. Neurodevelopmental abnormalities in megalin-, Shh- mice and Smo-deficient zebrafish

Tissue	Megalin-null phenotype*	Shh-null phenotype†	Smo-null phenotype‡
Neuroepithelium	Reduced telencephalon	Reduced forebrain and midbrain; loss of ventral forebrain structures	Failure to form parts of ventral forebrain
	Delayed anterior neural tube closure Displasia of forebrain neural folds Reduced epithelial cohesion	Absence of floor plate	Failure to form lateral floor plate
		Degeneration of caudal notochord	
	Pronounced cell death in regions facial acoustic, trigeminal, neural crest and optic vesicle	Abnormal dorsa/ventral patterning of neural tube	Pronounced cell death and decreased proliferation in CNS
Brain/spinal cord		Absence of the spinal column	
	Absence of olfactory bulbs	Absence of cranial motor axons	
		Absence of motor neurons	Failure to form secondary motoneurons
	Absence of corpus callosum		Disruption of forebrain axon tracts and commissures
	Fusion of forebrain hemispheres (holoprosencephaly) Prolapse of choroid plexus	Single continuous optic vesicle and fused telencephalon (holoprosencephaly)	
		Absence of diencephalon	Reduced hypothalamic tissue
	Exencephalus		Absence of pituitary
Eye	Microphthalmia or anopthalmia	Cyclopia Absence of optic stalks	Synophthalmia Absence of optic chiasma ectopic or expanded lenses
Nose	Shortened nose Dismorphology of frontonasal bones	Single fused nasal pit	

*Information was compiled from Willnow et al. (1996). †Information was compiled from Chiang et al. (1996) and Litingtung et al. (2000). ‡Information was compiled from Varga et al. (2001) and Chen et al. (2001).

embryonic epithelia mediates transepithelial transport of retinol to centers of active RA biosynthesis. If the first possibility was correct, megalin-deficient embryos would display a phenotype consistent with a retinol deficiency. However, embryos lacking retinol (Kastner et al., 1995), as well as those lacking Raldh2 (Niederreither et al., 2000), display neurodevelopmental phenotypes unlike those of megalin-deficient embryos (Willnow et al., 1996). Mice lacking dietary retinol or Raldh2 expression have severe hindbrain defects (Niederreither et al., 2000), whereas megalin-deficient mice display abnormalities in forebrain development (Willnow et al., 1996). The fact that megalin-deficient mice show no apparent hindbrain abnormalities indicates that VE transport of maternal retinol by megalin is not required to supply the embryo with retinol required for normal hindbrain patterning.

Early in development, embryonic requirements for retinol may be satisfied through simple diffusion-based transport; however, as embryonic epithelial integrity increases and/or epithelia become stratified, trans- and intra-epithelial transport mechanisms may be required. Since certain centers of RA expression are located adjacent to megalin-expressing epithelia, megalin might play a role in transport of retinol through or within stratified epithelial layers. In this way, it might mediate delivery of retinol to cells that express Raldh enzymes but are inaccessible to retinol. For example, sites of synthesis of RA by Raldh3 have been described in cells located deep in the basal layers of the ventral forebrain (Smith et al., 2001). Interestingly, suppression of Raldh3 expression in Pax6-deficient mice leads to microphthalmia and forebrain defects, including absence of olfactory bulbs (Suzuki et al., 2000), defects similar to those described for megalin-deficient mice (Willnow et al., 1996). It is thus possible that megalin expressed by cells of the apical layers of the forebrain and retinal epithelium (McCarthy et al., 2002) mediates transport of retinol to a subset of RA-synthesizing cells located in the basal regions of the developing forebrain and the retina. Similarly, megalin expressed on apical surfaces of the neural tube might facilitate the transport of retinol to subapical regions where retinoid-dependent neurons develop (Pierani et al., 1999).

Megalin and the regulation of hedgehog-dependent development

The hedgehogs are a family of proteins that act as morphogens during development, controlling cell growth, cell fate determination and organ patterning. The mouse has three hedgehog proteins: Indian hedgehog (Ihh), desert hedgehog (Dhh) and sonic hedgehog (Shh). Hedgehog proteins are synthesized as ~45 kDa precursors, which are cleaved to form N-terminal polypeptides (e.g. N-Shh) of ~25 kDa that have signaling activity (Lee et al., 1994; Porter et al., 1995). Developmental roles for Ihh include regulation of proliferation of cells during vasculogenesis, bone and cartilage formation and hematopoiesis (Ingham and McMahon, 2001). Roles for Dhh include control of gonad and germ line development and peripheral nerve sheath formation (Clark et al., 2000; Parmantier et al., 1999). Shh is perhaps the best-characterized member of the family (Ingham and McMahon, 2001). Concomitantly with autoproteolytic cleavage of Shh, cholesterol is covalently attached to its C-terminus, and palmitic acid is added to its N-terminus (Pepinsky et al., 1998; Porter et al., 1996). These lipid modifications are thought to be important for limiting the range of N-Shh signaling or targeting the protein to specific membrane domains (Bumcrot et al., 1995; Lee et al., 1994; Porter et al., 1995; Taylor et al., 1993). The current model of Shh signaling is that binding of N-Shh to patched (Ptc), a twelve-membrane-span protein containing a sterol-sensing domain, activates a second transmembrane protein, smoothened (Smo), which signals via a G-protein-coupled pathway (Ingham and McMahon, 2001).

During early development, Shh is prominently expressed in the notochord, the floorplate of the neural tube and the zone of polarizing activity in the limb (Marti et al., 1995). Numerous studies have demonstrated that Shh activity is critical for the induction, proliferation and survival of various embryonic cell types, including ventral neural cells in the developing brain, cranial neural crest cells and ventral neural retinal cells (Ingham and McMahon, 2001). For example, mice lacking Shh have anomalies of midline structures such as the notochord and floorplate of the early brain (Chiang et al., 1996) and later display an absence of ventral neuronal cells and cranial motor neurons (Litingtung and Chiang, 2000) (Table 1). The most common structural anomaly of the developing forebrain in Shh knockout mice is HPE. Mice lacking other components of the Shh signal transduction pathway also display neurodevelopmental abnormalities. For instance, mice lacking Ptc display embryonic lethality, but partial rescue of Ptc-null embryos results in severe exencephaly (Milenkovic et al., 1999) and a high incidence of cerebellar medulloblastomas characteristic of basal cell nevus syndrome (Goodrich et al., 1997). Smo-null zebrafish embryos show increased apoptosis of neural tube cells and other neural tube defects that include absence of secondary motor neurons, synopthalmia and ventral forebrain defects (Chen et al., 2001; Varga et al., 2001) (Table 1). Recent findings demonstrate that mice lacking dispatched (Disp), a protein critical to the secretion and long-range signaling of N-Shh, also display embryonic defects similar to those of $Shh^{-/-}$ and $Smo^{-/-}$ embryos (Ma et al., 2002). These include incomplete separation of the optic vesicles and a lack of a floorplate, resembling early anomalies in forebrain morphology that lead to HPE.

Since the spectrum of neurodevelopmental defects that make up the Shh-, Smo- and Disp-deficient phenotypes includes many key features of the megalin-deficient phenotype (Table 1), Herz and Bock have speculated that megalin is a component of the Shh signaling pathway (Herz and Bock, 2002). This speculation was fueled by studies showing that other members of the megalin family function as receptors for factors that influence neurodevelopment. For example, LRP6 functions as a co-receptor with frizzled to mediate signal transduction by the secreted signaling factor Wnt/Wg (Pinson et al., 2000; Tamai et al., 2000; Wehrli et al., 2000). Similarly, very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2) function as receptors for reelin, a protein that influences the positioning of dentate granule cells during brain development (Trommsdorff et al., 1999). Both megalin and LRP bind to midkine, a neurotrophic factor important for survival of embryonic neurons (Muramatsu et al., 2000). LRP has also been shown to mediate the endocytosis as well as the nuclear targeting of midkine, which is a requirement for its

ability to promote cell survival (Shibata et al., 2002). Recently, McCarthy et al. have found that megalin binds to the N-terminal fragment of Shh (N-Shh) (McCarthy et al., 2002). Furthermore, they have demonstrated that megalin functions as an endocytic receptor for N-Shh in cultured cells (McCarthy et al., 2002). These findings strengthen the idea that megalin influences Shh signaling in developing neural tissues, perhaps explaining the basis for many of the neurodevelopmental abnormalities observed in megalin-deficient embryos.

Thus far, a direct relationship between megalin-mediated endocytosis of Shh and regulation of Shh signaling has not been demonstrated. There are, however, several possible consequences of the interaction between megalin and Shh. The binding of N-Shh might elicit direct signaling by megalin, similar to the response of reelin binding to VLDLR and ApoER2, which activates phosphoinositide 3-kinase (PI 3-kinase) signaling (Beffert et al., 2002). Recent yeast two-hybrid screening studies demonstrate the potential of numerous cytoplasmic adaptor proteins to associate with the cytoplasmic tail of megalin (Gotthardt et al., 2000; Patrie et al., 2001; Petersen et al., 2003). This raises the possibility that megalin influences a variety of cell-signaling pathways. Interestingly, many of the adaptor proteins (e.g. Dab-1, JIP-1, MAGI-1 and PSD-95) have roles in neurogenesis and/or neuronal functions related to synaptic processes such as neurotransmitter receptor clustering and signaling complex assembly. Furthermore, several of the megalin-binding cytosolic proteins (e.g. JIP-1, Dab-2 and SEMCAP-1) regulate signal transduction pathways (Gotthardt et al., 2000). Recently, a novel cytosolic adaptor protein, MegBP, has been shown to bind to the PxxP motifs in the megalin cytoplasmic tail (Petersen et al., 2003). Since MegBP interacts with several transcriptional regulators (e.g. the SKI-interacting protein SKIP and TGF-β-stimulated clone 22 homologous gene (THG-1), it has been proposed that the megalin interaction with MegBP regulates the sequestration and release of MegBP-bound transcription factors (Petersen et al., 2003).

Although megalin might function as a Shh signaling receptor in its own right, the bulk of information on megalin function favors the hypothesis that it indirectly regulates Shh signaling through endocytosis of N-Shh. As stated above, megalin targets ligands to endosomes and transports ligands across epithelia. Several potential consequences of N-Shh interaction with megalin-expressing epithelial cells are depicted in Fig. 2. The interaction might not only mediate N-Shh uptake but also be part of an intracellular N-Shh trafficking system. A role for megalin in Shh trafficking is supported by evidence showing that the megalin–N-Shh interaction is resistant to dissociation at low pH and that N-Shh is not efficiently targeted to lysosomes for degradation in megalin-expressing cells (McCarthy et al., 2002). Avoidance of the lysosomes may then be part of a process by which N-Shh becomes targeted to specific cellular compartments, such as the basal or lateral surfaces of the epithelial cells for release. This would make N-Shh available to cells within the epithelium, thus extending the effective range of its signaling. Such a transcytotic, epithelial transport mechanism has been described for Wnt (Dierick and Bejsovec, 1998), which binds to the megalin family member arrow/LRP6 (Pinson et al., 2000; Tamai et al., 2000; Wehrli et al., 2000). This system appears to involve formation of Wnt-containing exovesicles called argosomes from basolateral membranes of imaginal disc epithelial cells and the subsequent endocytosis of these vesicles by adjacent epithelial cells (Greco et al., 2001). The process could establish a morphogen gradient by dispersing Wnt-containing membrane fragments over large distances through the *Drosophila* imaginal disc epithelium.

Another way in which megalin might influence Shh signaling is by modulating Ptc and Smo trafficking (Fig. 2). Several recent

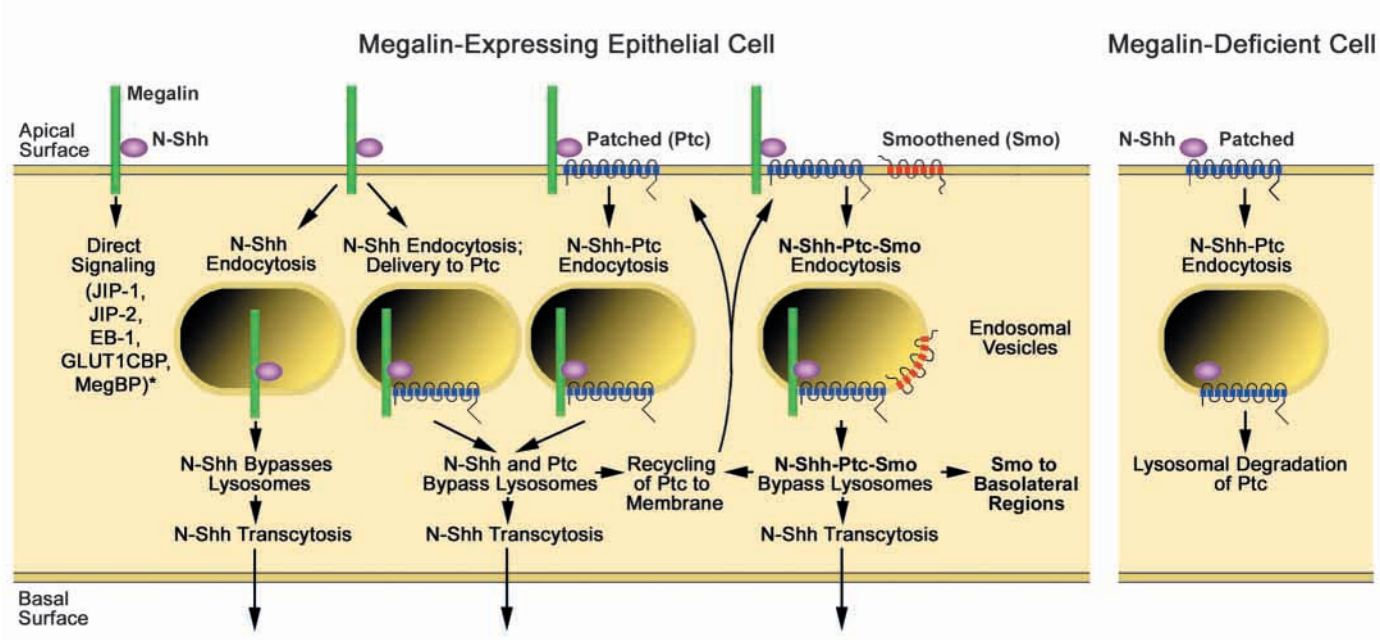

Fig. 2. Possible ways in which megalin might influence N-Shh trafficking and signaling. The mechanisms include direct signaling via megalin [the asterisk indicates megalin-binding adaptor proteins of the fetal brain (Petersen et al., 2003)], megalin-mediated endocytosis and trafficking of N-Shh leading to N-Shh transcytosis and megalin-mediated trafficking of the N-Shh receptors Ptc and Smo. Not depicted in the model are heparan sulfate proteoglycans, which have been shown to cooperate with megalin in N-Shh endocytosis (McCarthy et al., 2002).

studies indicate that there are low levels of Ptc on the surfaces of cells and that the bulk of Ptc protein exists in intracellular endosomal compartments (e.g. in imaginal disc epithelial cells) (Capdevila et al., 1994; Denef et al., 2000). Low cell surface levels of Ptc and increased endosomal accumulation may be the result of megalin-mediated endocytosis. This scenario would be analogous to the roles of LRP and VLDLR in mediating uptake of the urokinase receptor (uPAR) bound to urokinase-plasminogen activator inhibitor-1 complexes, as well as the uptake of the cell surface receptor tissue factor (TF) in complex with TF-pathway inhibitor and factor VIIa (Strickland et al., 2002). In each of these cases, the megalin family member regulates cell surface levels of the membrane receptors (uPAR and TF). Whether megalin can interact with Ptc is not known; however, it is possible that N-Shh serves as a ligand bridge similar to that in uptake of uPAR and TF by LRP and VLDLR. In support of this possibility are the findings of Incardona et al. showing that, after Shh binding, Ptc-Smo complexes are internalized in kidney epithelial cells (Incardona et al., 2002). Further, in wing disc epithelial cells that have responded to Shh, Ptc becomes concentrated in apical vesicles, whereas Smo becomes distributed basolaterally (Denef et al., 2000). These findings indicate that, following binding of N-Shh to epithelial cells, complex trafficking of Ptc and Smo occurs. Whether megalin orchestrates such trafficking in epithelial cells remains to be established.

Conclusion and Perspectives

Here, we have considered roles for megalin in neurodevelopment, emphasizing its involvement in the trafficking of and signaling by RA and N-Shh. Given that hindbrain development is dependent on maternally derived retinol, but that megalin-deficient embryos show no apparent hindbrain defects, a critical role for megalin in placental and VE uptake/transport of retinol required for proper neurodevelopment appears doubtful. One could test for the involvement of megalin in maternal-embryonic retinol transport by supplementing the diets of dams carrying megalin-deficient embryos with RA and looking for reduced severity in the neural phenotype of the null embryos. Perhaps the most plausible possibility is that megalin expressed by neuroepithelial cells plays a role in the transport of retinol to RA-generating sites located within the developing neuroepithelium that are involved with eye and forebrain development. Considering that embryos lacking megalin have neurodevelopmental abnormalities similar to those lacking genes of the Shh pathway, it is also reasonable to speculate that megalin is involved in epithelial trafficking of N-Shh. Such a role might be analogous to the epithelial trafficking of Wnt/Wg that is important for establishment of a signaling gradient in imaginal disc epithelium. In light of growing evidence that members of the LDL receptor family mediate signal transduction directly, the possibility that megalin mediates direct signaling in response to ligation with N-Shh must also be considered. Finally, given that several LDL receptor family members are known to influence surface levels of other membrane proteins, modulation of cell surface expression/trafficking of N-Shh receptors Ptc and Smo is yet another way in which megalin might impact N-Shh signal transduction during development.

References

Barth, J. L. and Argraves, W. S. (2001). Cubilin and megalin: partners in lipoprotein and vitamin metabolism. *Trends Cardiovasc. Med.* **11**, 26-31.

Beffert, U., Morfini, G., Bock, H. H., Reyna, H., Brady, S. T. and Herz, J. (2002). Reelin-mediated signaling locally regulates PKB/Akt and GSK-3beta. *J. Biol. Chem.* **277**, 49958-49964.

Buc-Caron, M. H., Condamine, H. and Kerjaschki, D. (1987). Rat Heymann nephritis antigen is closely related to brushin, a glycoprotein present in early mouse embryo epithelia. *Ann. Inst. Pasteur. Immunol.* **138**, 707-722.

Bumcrot, D. A., Takada, R. and McMahon, A. P. (1995). Proteolytic processing yields two secreted forms of sonic hedgehog. *Mol. Cell. Biol.* **15**, 2294-2303.

Capdevila, J., Pariente, F., Sampedro, J., Alonso, J. L. and Guerrero, I. (1994). Subcellular localization of the segment polarity protein patched suggests an interaction with the wingless reception complex in *Drosophila* embryos. *Development* **120**, 987-998.

Chen, W., Burgess, S. and Hopkins, N. (2001). Analysis of the Zebrafish smoothened mutant reveals conserved and divergent functions of hedgehog activity. *Development* **128**, 2385-2396.

Chiang, C., Litingtung, Y., Lee, E., Young, K. E., Corden, J. L., Westphal, H. and Beachy, P. A. (1996). Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature* **383**, 407-413.

Christensen, E. I., Moskaug, J. O., Vorum, H., Jacobsen, C., Gundersen, T. E., Nykjaer, A., Blomhoff, R., Willnow, T. E. and Moestrup, S. K. (1999). Evidence for an essential role of megalin in transepithelial transport of retinol. *J. Am. Soc. Nephrol.* **10**, 685-695.

Christensen, E. I. and Willnow, T. E. (1999). Essential role of megalin in renal proximal tubule for vitamin homeostasis. *J. Am. Soc. Nephrol.* **10**, 2224-2236.

Clark, A. M., Garland, K. K. and Russell, L. D. (2000). Desert hedgehog (Dhh) gene is required in the mouse testis for formation of adult-type Leydig cells and normal development of peritubular cells and seminiferous tubules. *Biol. Reprod.* **63**, 1825-1838.

Denef, N., Neubuser, D., Perez, L. and Cohen, S. M. (2000). Hedgehog induces opposite changes in turnover and subcellular localization of patched and smoothened. *Cell* **102**, 521-531.

Dierick, H. A. and Bejsovec, A. (1998). Functional analysis of Wingless reveals a link between intercellular ligand transport and dorsal-cell-specific signaling. *Development* **125**, 4729-4738.

Goodrich, L. V., Milenkovic, L., Higgins, K. M. and Scott, M. P. (1997). Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* **277**, 1109-1113.

Gotthardt, M., Trommsdorff, M., Nevitt, M. F., Shelton, J., Richardson, J. A., Stockinger, W., Nimpf, J. and Herz, J. (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**, 25616-25624.

Greco, V., Hannus, M. and Eaton, S. (2001). Argosomes. A potential vehicle for the spread of morphogens through epithelia. *Cell* **106**, 633-645.

Herz, J. and Bock, H. H. (2002). Lipoprotein receptors in the nervous system. *Annu. Rev. Biochem.* **71**, 405-434.

Incardona, J. P., Gruenberg, J. and Roelink, H. (2002). Sonic hedgehog induces the segregation of patched and smoothened in endosomes. *Curr. Biol.* **12**, 983-995.

Ingham, P. W. and McMahon, A. P. (2001). Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* **15**, 3059-3087.

Kastner, P., Mark, M. and Chambon, P. (1995). Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell* **83**, 859-869.

Kounnas, M. Z., Haudenschild, C. C., Strickland, D. K. and Argraves, W. S. (1994). Immunological localization of glycoprotein 330, low density lipoprotein receptor related protein and 39 kDa receptor associated protein in embryonic mouse tissues. *In Vivo* **8**, 343-351.

Lee, J. J., Ekker, S. C., von Kessler, D. P., Porter, J. A., Sun, B. I. and Beachy, P. A. (1994). Autoproteolysis in hedgehog protein biogenesis. *Science* **266**, 1528-1537.

Litingtung, Y. and Chiang, C. (2000). Control of Shh activity and signaling in the neural tube. *Dev. Dyn.* **219**, 143-154.

Ma, Y., Erkner, A., Gong, R., Yao, S., Taipale, J., Basler, K. and Beachy, P. (2002). Hedgehog-mediated patterning of the mammalian embryo requires transporter-like function of dispatched. *Cell* **111**, 63-75.

Marino, M., Andrews, D., Brown, D. and McCluskey, R. T. (2001).

Transcytosis of retinol-binding protein across renal proximal tubule cells after Megalin (gp 330)-mediated endocytosis. *J. Am. Soc. Nephrol.* **12**, 637-648.

Marti, E., Takada, R., Bumcrot, D. A., Sasaki, H. and McMahon, A. P. (1995). Distribution of Sonic hedgehog peptides in the developing chick and mouse embryo. *Development* **121**, 2537-2547.

McCarthy, R. A., Barth, J. L., Chintalapudi, M. R., Knaak, C. and Argraves, W. S. (2002). Megalin functions as an endocytic sonic hedgehog receptor. *J. Biol. Chem.* **277**, 25660-25667.

Means, A. L. and Gudas, L. J. (1995). The roles of retinoids in vertebrate development. *Annu. Rev. Biochem.* **64**, 201-233.

Mic, F. A., Haselbeck, R. J., Cuenca, A. E. and Duester, G. (2002). Novel retinoic acid generating activities in the neural tube and heart identified by conditional rescue of Raldh2 null mutant mice. *Development* **129**, 2271-2282.

Milenkovic, L., Goodrich, L. V., Higgins, K. M. and Scott, M. P. (1999). Mouse patched1 controls body size determination and limb patterning. *Development* **126**, 4431-4440.

Muramatsu, H., Zou, K., Sakaguchi, N., Ikematsu, S., Sakuma, S. and Muramatsu, T. (2000). LDL receptor-related protein as a component of the midkine receptor. *Biochem. Biophys. Res. Commun.* **270**, 936-941.

Niederreither, K., Vermot, J., Schuhbaur, B., Chambon, P. and Dolle, P. (2000). Retinoic acid synthesis and hindbrain patterning in the mouse embryo. *Development* **127**, 75-85.

Nykjaer, A., Dragun, D., Walther, D., Vorum, H., Jacobsen, C., Herz, J., Melsen, F., Christensen, E. I. and Willnow, T. E. (1999). An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell* **96**, 507-515.

Nykjaer, A. and Willnow, T. E. (2002). The low-density lipoprotein receptor gene family: a cellular Swiss army knife? *Trends Cell Biol.* **12**, 273-280.

Parmantier, E., Lynn, B., Lawson, D., Turmaine, M., Namini, S. S., Chakrabarti, L., McMahon, A. P., Jessen, K. R. and Mirsky, R. (1999). Schwann cell-derived Desert hedgehog controls the development of peripheral nerve sheaths. *Neuron* **23**, 713-724.

Patrie, K. M., Drescher, A. J., Goyal, M., Wiggins, R. C. and Margolis, B. (2001). The membrane-associated guanylate kinase protein magi-1 binds megalin and is present in glomerular podocytes. *J. Am. Soc. Nephrol.* **12**, 667-677.

Pepinsky, R. B., Zeng, C., Wen, D., Rayhorn, P., Baker, D. P., Williams, K. P., Bixler, S. A., Ambrose, C. M., Garber, E. A., Miatkowski, K. et al. (1998). Identification of a palmitic acid-modified form of human Sonic hedgehog. *J. Biol. Chem.* **273**, 14037-14045.

Petersen, H. H., Hilpert, J., Militz, D., Zandler, V., Jacobsen, C., Roebroek, A. J. M. and Willnow, T. E. (2003). Functional interaction of megalin with the megalin-binding protein (MegBP), a novel tetratrico peptide repeat-containing adaptor molecule. *J. Cell Sci.* **116**, 453-461.

Pierani, A., Brenner-Morton, S., Chiang, C. and Jessell, T. M. (1999). A sonic hedgehog-independent, retinoid-activated pathway of neurogenesis in the ventral spinal cord. *Cell* **97**, 903-915.

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.

Porter, J. A., von Kessler, D. P., Ekker, S. C., Young, K. E., Lee, J. J., Moses, K. and Beachy, P. A. (1995). The product of hedgehog autoproteolytic cleavage active in local and long-range signalling. *Nature* **374**, 363-366.

Porter, J. A., Young, K. E. and Beachy, P. A. (1996). Cholesterol modification of hedgehog signaling proteins in animal development. *Science* **274**, 255-259.

Rader, K., Orlando, R. A., Lou, X. and Farquhar, M. G. (2000). Characterization of ANKRA, a novel ankyrin repeat protein that interacts with the cytoplasmic domain of megalin. *J. Am. Soc. Nephrol.* **11**, 2167-2178.

Shibata, Y., Muramatsu, T., Hirai, M., Inui, T., Kimura, T., Saito, H., McCormick, L. M., Bu, G. and Kadomatsu, K. (2002). Nuclear targeting by the growth factor midkine. *Mol. Cell. Biol.* **22**, 6788-6796.

Smith, D., Wagner, E., Koul, O., McCaffery, P. and Drager, U. C. (2001). Retinoic acid synthesis for the developing telencephalon. *Cereb. Cortex* **11**, 894-905.

Springer, T. A. (1998). An extracellular beta-propeller module predicted in lipoprotein and scavenger receptors, tyrosine kinases, epidermal growth factor precursor, and extracellular matrix components. *J. Mol. Biol.* **283**, 837-862.

Strickland, D. K., Gonias, S. and Argraves, W. S. (2002). Diverse roles for the LDL receptor family. *Trends Endocrinol. Metab.* **13**, 66-74.

Suzuki, R., Shintani, T., Sakuta, H., Kato, A., Ohkawara, T., Osumi, N. and Noda, M. (2000). Identification of RALDH-3, a novel retinaldehyde dehydrogenase, expressed in the ventral region of the retina. *Mech. Dev.* **98**, 37-50.

Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J. P. and He, X. (2000). LDL-receptor-related proteins in Wnt signal transduction. *Nature* **407**, 530-535.

Taylor, A. M., Nakano, Y., Mohler, J. and Ingham, P. W. (1993). Contrasting distributions of patched and hedgehog proteins in the *Drosophila* embryo. *Mech. Dev.* **42**, 89-96.

Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R. E., Richardson, J. A. and Herz, J. (1999). Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* **97**, 689-701.

Varga, Z. M., Amores, A., Lewis, K. E., Yan, Y. L., Postlethwait, J. H., Eisen, J. S. and Westerfield, M. (2001). Zebrafish smoothened functions in ventral neural tube specification and axon tract formation. *Development* **128**, 3497-3509.

Wehrli, M., Dougan, S. T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A. and DiNardo, S. (2000). arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* **407**, 527-530.

Willnow, T. E., Hilpert, J., Armstrong, S. A., Rohlmann, A., Hammer, R. E., Burns, D. K. and Herz, J. (1996). Defective forebrain development in mice lacking gp330/megalin. *Proc. Natl. Acad. Sci. USA* **93**, 8460-8464.