The heterogeneous nature of congenital hydrocephalus has hampered our understanding of the molecular basis of this common clinical problem. However, disease gene identification and characterization of multiple transgenic mouse models has highlighted the importance of the subcommissural organ (SCO) and the ventricular ependymal (vel) cells. Here, we review how altered development and function of the SCO and vel cells contributes to hydrocephalus.

The cerebral spinal fluid (CSF) flow tract is a vital lifeline for supplying the brain with essential nutrients and growth factors throughout development and into adulthood. At the same time, the brain exercises constant control to ensure that the flow of CSF is homeostatic. CSF is secreted by the choroid plexus, failure to drain the CSF at the subarachnoid space, and the blockage of CSF flow through the confining canals of the ventricular system (Table 1).

The SCO is a small secretory gland positioned in the dorsal caudal aspect of the third ventricle underneath the posterior commissure. The SCO is derived from the neuroependymal cells that line the humps of the dorsal-caudal aspect of the diencephalon. This anatomic region is also described as the prosomere 1 (P1), demarcated by the pineal gland and the mesencephalon. The primitive epithelial progenitors of the presumptive SCO are driven toward a specialized secretory ependymal cell fate in response to the inducive Wnt and BMP signals emanating from the roof plate of the diencephalon boundary (FIGURE 1A).

Indeed, the roof plate acts as an important organizing center for the secretion of these morphogens that are crucial for establishing the dorsal-lateral identity of the neural plate of the diencephalon boundary (17, 38). Upon expression of Msx1 is observed at the neural fold stage, Msx2 and Msx3 form a family of homeodomain transcriptional repressors (18, 96, 111). The earliest expression of Msx1, a critical factor involved in dorsal neural patterning (3, 84). Msx1 along with Msx2 and Msx3 form a family of homeodomain transcriptional repressors (18, 96, 111). The earliest expression of Msx1 is observed at the neural fold stage, along the boundary of the neural plate (17, 38).

Upon closure of the neural tube, Msx1 is prominently expressed along the entire length of the dorsal midline of the neural tube. Msx1 is also expressed in the SCO, choroid plexus (CP), and the habenula in the third ventricle (84). Bach et al. clearly demonstrated that homozygous mutants for Msx1 were unable to sustain Wnt1 and BMP expression in the dorsal midline of P1, and histological analysis demonstrated the absence of a SCO and a poorly organized posterior commissure (3, 84). Loss of morphogen induction in P1 consequently downregulated cell fate markers such

The goal of this review is to summarize the molecular mechanisms that cause 1) the SCO to be absent or disorganized, 2) an inability of the SCO to properly secrete glycoproteins, 3) primary ciliary dyskinesia (PCD) of the ependymal cells, and 4) denudation of the neuroependyma. Although it should be noted that not all SCO/vel defects have been proven to precede the onset of hydrocephaly, indeed it is the aberrant execution of these diverse molecular pathways that can lead to stenosis of the aqueduct and contribute to communicating or non-communicating hydrocephalus.

**Loss of Developmental Factors Impairs SCO Formation and Function**

The SCO is small secretory gland positioned in the dorsal caudal aspect of the third ventricle beneath the posterior commissure. SCO is derived from the neuroependymal cells that line the humps of the dorsal-caudal aspect of the diencephalon. The primitive epithelial progenitors of the presumptive SCO are driven toward a specialized secretory ependymal cell fate in response to the inducive Wnt and BMP signals emanating from the roof plate of the diencephalon boundary (FIGURE 1A).

Indeed, the roof plate acts as an important organizing center for the secretion of these morphogens that are crucial for establishing the dorsal-lateral identity along the central nervous system (CNS) (31, 58, 59).

Although malformation of the SCO during gestation leads to ventricular dilation and aqueductal stenosis before birth (FIGURE 1B AND C), the involvement of specific factors has been slowly elucidated over the past decade through the analysis of mutant mouse models.

One such protein is Msx1, a critical factor involved in dorsal neural patterning (3, 84). Msx1 along with Msx2 and Msx3 form a family of homeodomain transcriptional repressors (18, 96, 111). The earliest expression of Msx1 is observed at the neural fold stage, along the boundary of the neural plate (17, 38). Upon closure of the neural tube, Msx1 is prominently expressed along the entire length of the dorsal midline of the neural tube. Msx1 is also expressed in the SCO, choroid plexus (CP), and the habenula in the third ventricle (84). Bach et al. clearly demonstrated that homozygous mutants for Msx1 were unable to sustain Wnt1 and BMP expression in the dorsal midline of P1, and histological analysis demonstrated the absence of a SCO and a poorly organized posterior commissure (3, 84). Loss of morphogen induction in P1 consequently downregulated cell fate markers such...
as Pax6, Pax7, and Lim1, whereas the P2 marker Glnz2 remained unaffected. The Mx1 mutant mouse developed hydrocephaly, and the interactions of Mx1 with Wnt1 and Pax6 are corroborated by similar hydrocephalic phenotypes in Wnt1<sup>–/–</sup> and Pax6<sup>–/–</sup> mice (27, 60).

It is also possible that Msx1 may regulate genes that maintain homeostasis in the mature SCO ependymal cells. Msx1 is already known to influence a diverse array of gene expression programs during neuronal development (85). Moreover, the residual ependymal cells of the SCO were not immunoreactive with anti-Reissner’s fiber serum (AFRU), suggesting the absence of the SCO were not immunoreactive with anti-Rfx3<sup>–/–</sup> mice, congenital non-communicating hydrocephaly was observed in the heterozygous condition (11). The agenesis of the SCO was ascertained by the degree of immunoreactivity was significantly reduced. Although both mouse knockout models resulted in the absence of a functional SCO, an intriguing observation was reported in the case of the Rfx phenotype. The Rfx<sup>–/–</sup> mice appeared to have an overall dysfuntional ependymal cell phenotype in forming or impinging on the ependymal properties of the Wnt7a-induced midbrain and a line defect was observed of Wnt7a and cilia formation along the dorsal midline of the Wnt, Brac2<sub>−/−</sub> mice to be potential players in this section.

### Table 1. Mutations that lead to hydrocephaly in the mouse

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description/Function</th>
<th>SCO/Neuroependymal Phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>En-1</td>
<td>Transcription factor</td>
<td>Ectopic expression leads to SCO agenesis, ependymal differentiation defects in CP/SCO</td>
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</tr>
<tr>
<td>Hdh</td>
<td>Unknown</td>
<td>SCO agenesis, ependymal differentiation defects</td>
<td>24</td>
</tr>
<tr>
<td>Max</td>
<td>Transcription factor</td>
<td>SCO agenesis, neuroependymal dysmorphology</td>
<td>3, 29, 84</td>
</tr>
<tr>
<td>Pax6</td>
<td>Transcription factor</td>
<td>SCO agenesis</td>
<td>27</td>
</tr>
<tr>
<td>Rfx3</td>
<td>Transcription factor</td>
<td>SCO agenesis, neuroependymal dysmorphology</td>
<td>2</td>
</tr>
<tr>
<td>Rfx4&lt;sup&gt;+&lt;/sup&gt;/3</td>
<td>Transcription factor</td>
<td>SCO agenesis, severe midline structure defects</td>
<td>11</td>
</tr>
<tr>
<td>Wnt1</td>
<td>Secreted morphogen</td>
<td>SCO agenesis</td>
<td>60</td>
</tr>
<tr>
<td>Pac1</td>
<td>G-protein coupled receptor</td>
<td>Overexpression leads to SCO agenesis, ependymal cilium is short and disorganized</td>
<td>50</td>
</tr>
<tr>
<td>Socs7</td>
<td>Suppressor of cytokine signaling</td>
<td>SCO cellular structure is disorganized</td>
<td>48</td>
</tr>
<tr>
<td>Hydin</td>
<td>Central pair-dynein adapter</td>
<td>Missing central pair projection; ciliary motility defects</td>
<td>22, 53</td>
</tr>
<tr>
<td>Foxf1/Hhex-H4</td>
<td>Transcription factor</td>
<td>Criogenesis defects</td>
<td>13, 20</td>
</tr>
<tr>
<td>Mdah5</td>
<td>Dynemin heavy chain</td>
<td>Outer dynein arms missing, ciliary motility defects</td>
<td>39, 41</td>
</tr>
<tr>
<td>Pcpd1</td>
<td>Unknown; may bind to central pair</td>
<td>Ciliary motility defects</td>
<td>55</td>
</tr>
<tr>
<td>Pola2/If188</td>
<td>IFT particle protein</td>
<td>Epithelial cells are shorter, disorganized, and beat asynchronously; CP defects</td>
<td>6</td>
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<tr>
<td>Pola2</td>
<td>DNA repair polymerase</td>
<td>Ciliary motility defects</td>
<td>47</td>
</tr>
<tr>
<td>Spag6</td>
<td>Central pair-dynein adapter</td>
<td>Ciliary motility defects, Spag16 inactivation increases severity of hydrocephalus</td>
<td>93, 116</td>
</tr>
</tbody>
</table>

**Developmental defects**

**Cilia structure/function defects**

**Cell adhesion defects**

*Unless otherwise stated, all mutations are inactivating.*

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*Tables, figures, and references are not included in this response.*

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immunoreactive in the adult (2, 11). In both cases, a functional SCO, an overall dysfunction in the development of the ciliated ependymal cells. More specifically, the CP was poorly formed or improperly differentiated, with a lack of cilia present on the apical surface. Furthermore, the cell adhesion properties of the CP ependymal cells were compromised since the interdigitations between cells were poorly formed and the basal membrane was not apposed to the basal lamina. This apparent lack of differentiation was also observed in the SCO where its ependymal cells were more cuboidal with many cilia, appearing similar to the ependyma lining the ventricles (2).

In the case of the Rfx4_v3 studies, homozygous knockouts of Rfx4_v3 strongly suggest its role as a critical mediator of an earlier dorsal midline patterning decision. Rfx4_v3–/– mice were embryonic lethal due to catastrophic midline defects. Rfx4_v3–/– telencephalon at E12.5 were characterized by hypoplasia of the dorsal midline and adjacent cerebral cortex. The dorsal midline defect was also evident due to the down regulation of Wnt7a and Wnt7b in the cortical hem, a midline structure that normally produces these transcripts (11). A recent microarray experiment identified components of the Wnt, Bmp, and retinoic acid signaling pathways to be potential targets of Rfx4_v3 (10).

From the studies described above, tight regulation along the dorsal midline through Wnt and Bmp signaling pathways is an important regulatory mechanism for the proper development of the differentiated ependymal cells of the SCO and for preventing the onset of hydrocephalus. Indeed, this is also noted in mice that ectopically express Engrailed-1 (En-1), a transcription factor that is important for establishment of the mid-hindbrain boundary, since these animals were presented with hydrocephaly and SCO agenesis in the P1 domain (60). Similarly, a recent report by Dietrich et al. produced a mouse model linking the Huntington’s disease gene (Hdh) to congenital hydrocephaly (24). In this study, a mid- to hindbrain-specific inactivation of Hdh was generated by crossing Hdhlox/lox mice with animals that express Cre under the control of a Wnt1 regulatory element. The Wnt1-Cre;Hdhlox/lox mice came to term but displayed reduced growth and progressive wasting due to the build up of excessive CSF, expansion of the ventricles, and the loss of cortical tissue. Histology revealed expansion of the lateral ventricles by E17.5, indicating the congenital nature of the hydrocephaly. Despite the complete absence of huntingtin (ht) in the mutant CP, no gross structural defects were observed using immunohistochemistry for a variety of markers (24). The effect of hit loss was more overt in the mutant SCO. Mutant SCO was ~40% the size of the controls. Serial coronal

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sections revealed that only the rostral portion of the SCO appeared properly differentiated as detected by SCO-spondin staining. Moreover, caudal to the SCO domain, ectopic SCO-spondin expression was detected in the ependymal lining of the Sylvian aqueduct (24). Further characterization of these mice is needed to determine whether the defect lies in inappropriate secretion of glycoproteins from the SCO or altered function of the vel cells lining the aqueduct. Nonetheless, these findings suggest that htt has a role in regulating the function of specialized ependymal cells. Taken together, the proper development of the SCO is essential for CSF circulation through the narrow Sylvian aqueduct, and other factors that regulate dorsal patterning at or near the mid-hindbrain boundary are potential candidates for genetic causes of congenital hydrocephaly. In this regard, a protein like Snf2l, which modulates chromatin structure and expression of En-1, may potentially have dire consequences for hydrocephaly (8). Although we are only beginning to unravel the multitude of factors that control the cell fate decisions in this region and the terminal differentiation of the specialized cells of the SCO and vel, it is apparent that there is extreme heterogeneity underlying hydrocephalus. It should also be mentioned that other transcription factors, such as Otx2, are known to be associated with hydrocephalus when inactivated in mice; however, such a phenotype is unlikely to be SCO-specific and likely constitutes a more general defect in brain development (62). Although development seems to have a significant role in the etiology of hydrocephalus, aberrant function of the SCO and vel cells has also been implicated in this disease.

**Signal Transduction and the Regulation of SCO Secretion**

The primary function of the SCO is to secrete high molecular weight glycoproteins that facilitate CSF flow and, in rodents, contribute to the formation of Reissner's fiber (RF), which extends along the length of the CSF tract to the ampulla caudalis (89). The major secreted glycoprotein and constituent of RF is SCO-spondin, a highly conserved protein containing many protein domains that fostered its role as a neuronal pathfinding molecule. In mice, its precise function has been shown to be critical for normal development of the CNS (99). Although terminal differentiation of the SCO is essential for CSF circulation through the narrow Sylvian aqueduct, and other factors that regulate dorsal patterning at or near the mid-hindbrain boundary are potential candidates for genetic causes of congenital hydrocephaly. In this regard, a protein like Snf2l, which modulates chromatin structure and expression of En-1, may potentially have dire consequences for hydrocephaly (8). Although we are only beginning to unravel the multitude of factors that control the cell fate decisions in this region and the terminal differentiation of the specialized cells of the SCO and vel, it is apparent that there is extreme heterogeneity underlying hydrocephalus. It should also be mentioned that other transcription factors, such as Otx2, are known to be associated with hydrocephalus when inactivated in mice; however, such a phenotype is unlikely to be SCO-specific and likely constitutes a more general defect in brain development (62). Although development seems to have a significant role in the etiology of hydrocephalus, aberrant function of the SCO and vel cells has also been implicated in this disease.

**FIGURE 2. Molecular pathways in ependymal cells implicated in congenital hydrocephalus**

Schematic representation of a SCO/vel ependymal cell highlighting the numerous molecular pathways underlying congenital hydrocephalus. Neuropeptides (Pacap, serotonin) induce cell signaling pathways that alter target gene regulation, presumably genes encoding secreted glycoproteins and proteins involved in cilary function. The Socs7 protein is a regulator of the Jak/Stat signaling pathway. Ablation of several transcription factors (Msx1, Rfx3, Rfx4, v3) alters the terminal differentiation or cell fate of the ependymal cells. Numerous proteins (Midn4h5, Hydro, Prq/sp, Spag7, Polaris) and transcriptional regulators (Foxj1/4/6-4, Pol 4) involved in the formation and function of cilia are implicated in hydrocephalus and are shown at the top of the diagram. The α-Snap protein is involved in regulating the transport of cell adhesion molecules (N-myc, cadherin) to the apical cell surface and presumably also for proper glycoprotein secretion.
Many protein-protein interaction domains that have fostered its numerous putative functions, including axonal pathfinding, promoting neuronal survival and neurite extension, and a detoxifying activity by removing monoamines from the CSF (67, 68). Regardless of its precise function, alterations in SCO secretions have been shown to precede diagnoses of hydrocephalus (99). Although transcription factors that promote terminal differentiation are important for regulating the expression of secreted proteins (e.g., Msx1) from the SCO, increasing evidence implicates signal transduction mechanisms in the processes regulating SCO secretion as a cause of hydrocephalus (Figure 2). The importance of signaling pathways downstream of G-protein-coupled receptors (GPCR) (73) in particular may modulate SCO glycoprotein secretion through the induction of transcriptional regulators, such as CREB, and perhaps also through the induction of intracellular calcium levels (18, 50, 74, 81, 94, 95). Recently developed hydrocephalic transgenic mouse models (Pac1, Ro1) seem to indicate that GPCR concentrations are rigidly controlled (50, 100).

The PACAP type I (Pac1) receptor is one example of a GPCR that contributes to hydrocephaly (50). Murine Pac1 is highly expressed in neural progenitor cells, and transgenic Pac1 was also found to be highly expressed in the differentiated SCO and ventricular ependyma (50, 106). Pituitary adenylate cyclase-activating polypeptide (PACAP) is the primary Pac1 ligand, and its interactions with Pac1 have been shown to activate or inhibit a variety of downstream signaling pathways, including PKA, PKC, MAPK, RhoA, and IP3 (Figure 2) (66). Transgenic mice that overexpressed Pac1 developed communicating hydrocephalus with an enlarged aqueduct, which was associated with apoptosis-related SCO agenesis and shortened disorganized ventricular cilia (50). The mutant phenotype was correlated with an activation of both PKA and PKC signaling; however, the impact on SCO secretion was not assessed.

Neurotransmitting hormones are some of the most well characterized ligands of GPCRs, and the SCO is well innervated by serotonergic, GABAergic, dopaminergic, and noradrenergic fibers (42). Serotonin and GABA have also been previously characterized as regulators of SCO secretion; however, the molecular mechanisms have not been well explored (56, 70, 92). The most recent molecular studies concerning the effect of neurotransmitters on SCO secretion demonstrate a significant amount of serotonin and dopamine receptors on the apical surface of the mammalian SCO ependyma, implicating the CSF as the primary source of their respective ligands (97, 103). Although dopamine signaling did not have any significant effect on SCO secretion, serotonin signaling through the 5HT2A receptor, which primarily stimulates the phospholipase C (PLC) pathway, was shown to inhibit the transcription of SCO-spondin. To date, defects in serotonin signaling have not been identified as a primary cause of hydrocephalus. However, a significant reduction in the rate of serotonin turnover has been observed in the CSF of human infants suffering from hydrocephalus (34), which may account for secondary defects in SCO secretion that could contribute to a worsening of the disease.

“Recent molecular evidence in humans indicates that the direction of cell adhesion molecules to the proper surface is vital.”

Abnormal cytokine and growth factor signaling pathways have also been linked with hydrocephaly (reviewed in Ref. 112). One study has shown that suppressor of cytokine signaling 7 (Socs7) mutant mice develop late-onset communicating hydrocephaly, where the only observable physiological abnormality is a disorganized SCO (48). Abnormalities in SCO secretion and RF formation were not examined. SOCS proteins are traditionally considered to suppress cytokine signaling by binding to JAK receptors to prevent STAT transduction into the nucleus (51). Although this may be the role of Socs7 in SCO cells, the protein has also been implicated in several nontraditional transduction roles, including its binding to phosphorylated STATs (63), binding to other SOCS proteins (82), binding to the actin cytoskeleton through interactions with septins or vextin (46, 49, 64), or contributing to the regulation of the actin cytoskeleton and cell cycle mechanisms by shutting NCK to the nucleus (46, 49). At this time, the molecular significance of Socs7 with respect to the SCO remains to be characterized.

Taken together, these studies highlight the possibility that constituents of the CSF feedback on the SCO to regulate glycoprotein production and/or secretion. Although disruptions in specific signaling pathways have not yet been proven to be a primary cause of hydrocephaly, it represents an exciting area for future studies that would provide an additional layer of complexity to our understanding of the causal mechanisms of congenital hydrocephalus and SCO function.

The Contribution of Ventricular Cilia to CSF Homeostasis

The ventricular ependyma shares complementary functions with the SCO in regulating CSF homeostasis and preventing the onset of hydrocephalus (81). Besides the requirement for SCO glycoproteins and RF to prevent stenosis of the Sylvian aqueduct, a unidirectional laminar flow of CSF generated by the ciliated ependyma is essential (41).
Hydrocephalus in humans arises in several clinical syndromes, which are characterized by defects in cilia structure and function (4, 30, 40, 109). In the ventricular ependyma, the ciliary core, or axoneme, consists of an arrangement of a central pair of microtubules surrounded by nine peripheral microtubule doublets. Radial spokes project from the central pair and interact with the peripheral doublets, presumably to regulate interactions with dyneins, which are motor proteins that are responsible for generating ciliary beat (91).

Mice and humans with mutations in IFT88, IFT122, or DNAH5 have a reduced beat frequency due to the absence of outer dynein arms in the axoneme (39). Its human counterpart, DNAH5, has been identified in the absence of outer dynein arms in the axoneme of Hydrocephalus cephalus (37, 75).

The molecular characterization of the hydrocephalus-3 (hy3) mouse, a commonly studied model of hydrocephalus, resulted in the identification of the Hydin gene (22). Hydin is a putative microtubule binding protein (83) required for the formation of a specific projection emanating from one of the central pair microtubules (23, 53, 54). Lechtreck et al. believe that Hydin is a dynein adaptor that is required to regulate the transition between the ciliary effective and recovery strokes (53). This proposal is consistent with observations that the ependymal cilia of hy3 mice have difficulty bending, leading to ciliary beats with reduced frequency and impaired synchrony (53). At the same time, these cilia do not appear to have any structural defects other than the lack of this single central pair projection. The authors note that the phenotype is similar to that of PCD patients who suffer defects in the inner dynein arms or the radial spokes (21, 53).

Sperm-associated antigen 2 (Spag17) is another factor that associates with a specific central pair projection and, along with its interacting partners Spag16 and Spac17, is required for ciliary motility (83, 113-115). Like Hydin, Spag17–/– mutants suffer from hydrocephalus. The extent of the phenotype is made more severe when both Spag16 and Spag17 are knocked out, although Spag16–/– mice are not hydrocephalic (116). The ultrastructure of the cilia in these animals appears normal, suggesting a molecular pathology that is similar to hy3 mice. Moreover, Spag26 has been described as a candidate gene for hydrocephalus in H-Tx rats, another commonly studied hydrocephalus model (43).

PCD protein 1 (Pcdp1) is another putative central pair-binding protein that, when ablated in mice, results in hydrocephalus (55). As with hy3 and Spag6–/– mice, the tracheal cilia of the Pcdp1–/– mice have a reduced beat frequency but do not display any obvious ultrastructural anomalous defects. The ultrastructure and ciliary beat frequency of epndymal cilia were not reported, although Pcdp1 was identified throughout the cilia of human brain epndymal cells.

In the Tg3371 or pk mouse (57), mutations in the intrahelical transport protein (91) Polaris/HRB8 also led to hydrocephalus. Although epndymal cilia are shorter, disorganized, and beat asynchronously, the cause of hydrocephalus in these mice appears to be the regulation of intracellular and secretory mechanisms of the choroid plexus in response to the defects in the structure and function of its cilia (5, 6). IFT components appear to be well conserved and many await characterization, so it remains to be seen whether mutations in additional IFT factors also result in hydrocephalus. Defects in the expression of transcriptional regulators that mediate ciliogenesis can also lead to hydrocephalus. For example, FoxJ1/HH-4 is an important transcriptional activator of numerous genes required for the development of motile cilia and has been associated with left-right asymmetry and developmental defects and associated hydrocephaly (13, 20, 108). Similarly, mice that lack DNA polymerase λ (Pol λ) also develop hydrocephalus (47). Although Pol λ is generally considered to be a DNA repair enzyme, it is required for the proper expression of the axonemal inner dynein arms whose dysfunction likely contributes to the hydrocephaly in these animals (33).

In this section, we have highlighted recent advances characterizing defects in cilia structure and function that result in hydrocephalus. It is likely that additional proteins that interact with or comprise the cilia ultrastructure are potential candidates to be implicated in hydrocephalus in the near future.

Cell Adhesion Molecules and Hydrocephalus

The ependymal cells of the SCO possess tight junctions and zonular adherens, which contribute to the formation of a blood-brain barrier between the vascular and the ventricular cavities (89). Adding to the complexity of its functions is accumulating evidence that the SCO is able to act as a mechanosensor, altering the way it secretes glycoproteins in response to signals sent from circulating blood, as shown in hypertensive rats (65). In this regard, it is intriguing that hypertension was also observed in both Pac1-null and Pcdp1–/– mice. Regardless, the presence of such a blood-brain barrier necessitates a functional complement of cell adhesion molecules.

Recent molecular evidence in humans indicates that the direction of cell adhesion molecules to the proper surface is vital. Mutations in the vesicular transport protein 18 (VPS18) cause periventricular reduction in ependyma, Hydrocephalus cephalus (hyh) model, and hop gait (hyh) (105). Ependymal cilia, identified in cell culture, are motor proteins that are responsible for generating ciliary beat (91), while cell surface molecules on ependymal cells form a meshwork of integral membrane proteins (19). It is suggested that the meshwork is involved in the transport of glycoproteins out of the hydropropid and into the cerebrospinal fluid (CSF) (184). This proposal is consistent with observations that the ependymal cilia of hy3 mice develop hydrocephalus. The extent of the phenotype is made more severe when both Spag16 and Spag6 are knocked out, although Spag6–/– mice are not hydrocephalic (13, 20, 108). Similarly, mice that lack DNA polymerase λ (Pol λ) also develop hydrocephalus (47). Although Pol λ is generally considered to be a DNA repair enzyme, it is required for the proper expression of the axonemal inner dynein arms whose dysfunction likely contributes to the hydrocephaly in these animals (33).

In the Tg3371 or pk mouse (57), mutations in the intrahelical transport protein (91) Polaris/HRB8 also led to hydrocephalus. Although epndymal cilia are shorter, disorganized, and beat asynchronously, the cause of hydrocephalus in these mice appears to be the regulation of intracellular and secretory mechanisms of the choroid plexus in response to the defects in the structure and function of its cilia (5, 6). IFT components appear to be well conserved and many await characterization, so it remains to be seen whether mutations in additional IFT factors also result in hydrocephalus. Defects in the expression of transcriptional regulators that mediate ciliogenesis can also lead to hydrocephalus. For example, FoxJ1/HH-4 is an important transcriptional activator of numerous genes required for the development of motile cilia and has been associated with left-right asymmetry and developmental defects and associated hydrocephaly (13, 20, 108). Similarly, mice that lack DNA polymerase λ (Pol λ) also develop hydrocephalus (47). Although Pol λ is generally considered to be a DNA repair enzyme, it is required for the proper expression of the axonemal inner dynein arms whose dysfunction likely contributes to the hydrocephaly in these animals (33).

In this section, we have highlighted recent advances characterizing defects in cilia structure and function that result in hydrocephalus. It is likely that additional proteins that interact with or comprise the cilia ultrastructure are potential candidates to be implicated in hydrocephalus in the near future.

Cell Adhesion Molecules and Hydrocephalus

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transport proteins FILAMIN A and ARFGEF2 lead to periventricular heterotopia, which is associated with a reduction in cell adhesion and a loss of the neuroependyma, and is sometimes accompanied by hydrocephalus (28, 77, 98). In the hydrocephalus with hop gait (hyh) mouse, hydrocephalus is accompanied by a denudation of the ependymal cell layer (14, 79, 105). Ependymal denudation of the SCO has been identified in cases of human hydrocephaly (25).

A mutation in the soluble NSF attachment protein α (α-Snap), which directs secretory vesicles to the apical surface of neural progenitors, is responsible for the hyh phenotype (19, 36). α-Snap is known to direct cell adhesion molecules, such as E-cadherin and β-catenin, to the apical surface of neural progenitors (9, 19, 76). In vel cells, non-muscle myosin II-B heavy chain (Nmhc-IIb) forms a mesh-like structure with N-cadherin and β-catenin at the apical surface, thus it is possible that α-Snap is involved in the localization of one or more of these structural components (FIGURE 2) (61). Similar to the hyh phenotype, the loss of Nmhc-IIb results in a loss of integrity in the neosophematum, leading to hydrocephalus and defects in neural cell migration (61, 104). This phenotype can be somewhat rescued by replacing the expression of Nmhc-IIb with Nmhc-IIa (7).

**Mouse-Human SCO Differences and the Relationship to the Human Condition**

CNS patterning is a highly conserved process underpinned by fundamental molecular pathways and interactions in all vertebrates. Moreover, select classes of transcription factors such as the HMLH, homeobox, and paired box family are key regulators of CNS development in metazoans. Taken together, it suggests that mouse models represent a powerful system in which to understand the etiology of hydrocephaly in humans. Indeed, the presence of the SCO/vel in even the earliest of vertebrates suggests a high degree of conservation and function in these tissues, and one might predict that most of the factors identified in mice to cause human hydrocephaly are present in only a small number of these patients (61). Similar to its ligand, PACAP, is located at 18p11, and congenital hydrocephalus is one of the symptoms experienced by patients with tetrasomy 18p (102). Similarly, HYDIN, located at 16q22, is a candidate for one case of human hydrocephaly (16). A large-scale genetic screen has also recently revealed that mutations in a human-specific HYDIN paralog located at 1q21 may contribute to a number of behavioral and congenital brain disorders, although hydrocephalus was present in only a small number of these patients (15). Additional comparisons between human and mouse are summarized in a recent review of the genetics of human hydrocephaly (112).

Although we have highlighted a role for many factors in the etiology of hydrocephaly in the mouse, human studies identifying causative genes are lagging far behind. One reason for this delay may evolve from the general nature of hydrocephaly itself. It is a very heterogeneous disorder and without the availability of large families for genetic studies it is difficult to group patients together for successful linkage analyses. Another consideration is that many of the animal models represent a powerful system in which to further characterize the similarities and differences between the two species in disease development. In this regard, L1-CAM, an X-linked cell adhesion molecule important for neurite outgrowth, is the best characterized determinant of congenital human hydrocephaly, and mouse models have been shown to accurately recapitulate the human phenotype (44, 72, 90, 107). Overall, a combination of animal and human studies remains the best approach for disease gene identification.
Concluding Remarks

Collectively, these studies demonstrate that defects in multiple aspects of SCO cell function are implicated in the development of congenital hydrocephalus. As such, the new front for SCO research is clearly toward a further understanding of the molecular mechanisms governing development and function. The plethora of genetic mouse models available is phenomenal and should continue to be extremely valuable for researchers in this field and in the hunt for causes of human hydrocephalus.

Developing studies will continue to elucidate the hierarchical transcription factor organization that dictates the stage-specific differentiation of these cells and underlies the critical points of functional regulation. Defining these pathways will present researchers with candidate disease genes and opportunities to augment differentiation of progenitors toward an SCO fate to generate cell culture models. Similarly, with a complete understanding of the signaling mechanisms between the CSF and SCO cell vesicles should identify ligands that could be administered to promote cilia protein secretion and restore CSF homeostasis. Finally, the use of high throughput approaches will enhance the knowledge of the important protein-protein interactions. For example, proteomic approaches have been used in the initial characterization of the secreted glycoproteins from the SCO and, more generally, for delineating the cilary phenotype (78). Interestingly, this proteomic study identified more than 600 proteins, of which over 200 were conserved between humans and Chlamydomonas, including a surprising number of proteins involved in signal transduction mechanisms (78). All of these approaches will increase our knowledge of SCO function and help elucidate where similarities to human hydrocephaly exist, thereby facilitating the characterization of genetic causes of congenital hydrocephalus.

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