

SA1

Stem cells and the organisation of the gut

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Stem cells are primitive cells located in a specialised mesenchymal “niche” that lack expression of any definitive markers of lineage commitment and are therefore difficult to define and identify. Stem cells maintain their capacity for limitless self replication throughout the lifetime of their host, and can also divide to produce daughter cells, committed to the formation of every adult cell lineage within their tissue of origin. The stem cells of the gastrointestinal tract remain unidentified which has led to many conflicting hypotheses as to their precise nature and function. For example, the numbers and location of stem cells in the intestinal crypts and gastric glands have never been conclusively proven and, consequently, the clonal origins of these structures under normal circumstances and in neoplasia are clouded issues. The morphological events of gastrointestinal carcinoma formation are hotly debated, with two main conflicting hypotheses of the mechanisms of expansion of a mutated stem cell clone. However, with the emergence of the molecular pathways governing gastrointestinal stem cell function, and the identification of putative intestinal molecular stem cell markers, such as Musashi-1, comes a clearer insight into the properties of the gastrointestinal stem cell. Adult stem cells from several tissues can leave their niche and engraft into extraneous tissues, including the gastrointestinal mucosa and underlying mesenchyme, and transform to produce adult cell lineages common to these foreign environments. This process is optimal when the requirement for regeneration is enhanced (that is, in diseased or damaged tissue) and indeed, the contribution of transplanted bone marrow stem cells to intestinal myofibroblasts is significantly upregulated in colitis. However, adult stem cell plasticity has recently been disparaged by reports suggesting that stem cells spontaneously fuse with indigenous adult cells to form a diploid cell with an aberrant karyotype, and it is important to investigate if bone marrow cells contribute to a gastrointestinal stem cell population, and indeed the mechanisms by which they do so. Identification of the origins, location, and molecular regulators of the gastrointestinal stem cell will provide a clearer understanding of normal gastrointestinal function and the genetic pathways involved in neoplastic change.

Humphries A, Wright NA. Colonic crypt organization and tumorigenesis. *Nat Rev Cancer*. 2008 Jun;8(6):415-24. Epub 2008 May 15.

Leedham SJ, Brittan M, McDonald SA, Wright NA (2005) Intestinal stem cells. *J Cell Mol Me* 9: 11-24

M Brittan, NA Wright Stem cells in gastrointestinal structure and neoplastic development *Gut* 2004;53:899-910

This work was supported by Cancer Research UK

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SA2

Conversion of pancreas to liver

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Transdifferentiation (or sometimes it is referred to as metaplasia) is defined as the conversion of one cell type to another. Cells which undergo transdifferentiation generally arise from adjacent regions of the developing embryo. For example, liver and pancreas arise from the same region of the endoderm. Understanding transdifferentiation is important to developmental biologists because it will help elucidate the cellular and molecular differences that distinguish neighbouring regions of the embryo. To address this issue, we have developed models for the conversion of pancreatic cells and hepatocytes. Addition of the synthetic glucocorticoid dexamethasone is sufficient to induce a hepatic phenotype in pancreatic cells (the rat pancreatic cell line AR42J-B13, embryonic mouse pancreas and human foetal pancreatic cells). The hepatocytes induced by transdifferentiation express a mature liver phenotype and are able to perform at least some of the functions of bone fide hepatocytes. We have determined the molecular basis of the switch from pancreatic to the hepatic phenotype and found that the transcription factor CCAAT enhancer binding protein beta (C/EBPbeta) is able to induce a hepatic phenotype when overexpressed in pancreatic cells. Expression analysis of members of the C/EBP family demonstrates liver but not pancreas expression during embryonic development. C/EBPs may therefore distinguish pancreas and liver tissues during embryonic development.

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SA3

Nephronophthisis – disease mechanisms of a ciliopathy

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Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disorder. Recent molecular genetic advances have allowed identification of several genes underlying NPHP. NPHP genes encode proteins known as nephrocystins which are located in the primary cilium, basal body complex, adherens junction and focal adhesion kinase protein complexes. This shared localisation has led to a paradigm whereby all genes mutated in cystic kidney diseases express their protein products in the primary cilium and/or basal body structures, suggesting that common pathogenic mechanisms underlie this multisystem disease. Cilial proteins are highly conserved throughout evolution and their defects lead to an expanding group of conditions known as ciliopathies.

Functional studies implicate nephrocystins in planar cell polarity pathways, which may be crucial for renal development and

maintenance of tubular architecture. Mechanisms underlying cystogenesis and therapeutic interventions are being explored in model systems enabling new insights into this disease.

Sayer JA et al. (2006). The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. *Nat Genet* 38, 674-681.

Simms RJ et al. (2009). Nephronophthisis. *Eur J Hum Genet* 17, 406-416.

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SA4

Branching morphogenesis: a closer view using live imaging

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The major aim of our research is to gain a better understanding of how epithelial organs reach and maintain their three-dimensional shape. The underlying morphogenesis processes are regulated by cellular interactions and by the developmental histories of the participating cells. To study cell signalling, we concentrate on two signalling pathways, the Dpp/Bmp and the Fgf pathway. To study the process of morphogenesis, we use the developing tracheal network of the fly and the vascular network of the zebrafish as a model system. Both tissues arise in part through a process called branching morphogenesis, which turns epithelial sheets into tubular networks with distinct features regarding the size, the length and the function of tubes. The branching process is controlled by cell signalling and cell interactions but the consequences of these latter in the responding cells are often poorly understood. Over the years, we have undertaken major efforts to use live imaging in combination with laser ablation to better understand these fascinating morphogenesis processes.

We have characterized in detail cell rearrangements during tracheal tube formation in the fly using a GFP fusion protein labelling the Adherens Junctions (AJ) combined with high resolution live imaging, both in wild type and in mutant embryos. To better analyse the branching process at the cellular level, we also developed a single cell imaging approach, and used it to follow wild type or mutant cells during branch formation. We find that complex processes of cell intercalation, which require extensive AJ remodelling, are involved in the formation of branches of different cellular complexities in the trachea, and propose a model of how these cell intercalations occur. We have subsequently used this model to better understand defects in distinct tubes in a number of mutant situations, and tried to define the forces involved in intercalation. These studies attributed important roles for cell signalling in regulating transcription factors; these latter eventually control cell behaviour with regard to cell rearrangements. In addition, our studies point to important roles for apically secreted, luminal proteins in the tubulogenesis process.

More recently, we have also started to analyze angiogenesis in zebrafish using similar approaches, with particular emphasis

on vessel sprouting and vessel fusion. First interesting results of these studies will also be presented.

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SA5

Signaling to the Cytoskeleton by pH Sensing

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Increasing evidence indicates that changes in intracellular pH (pHi) regulate cell morphology and cytoskeleton organization. However, our understanding of how pHi affects protein conformations and macromolecular assemblies to regulate cytoskeleton dynamics and other cell behaviors is limited. We are determining design principles, structural features, and functions of pH sensors or proteins that have activities or ligand-binding affinities that are sensitive to small, physiologically relevant changes in pH. Protonation is the simplest possible electrostatic switch for modulating protein function. And in contrast to other posttranslational modifications or cofactors, protonation can confer extremely rapid temporal responses and the modulation of multiple proteins in unison to control a cell behavior. Our current work bridges structural and cellular biology to elucidate at the molecular level how pHi regulates cytoskeletal proteins conferring cell polarity, actin filament assembly, and cell-substrate adhesion remodeling. These processes control distinct stages in directed cell migration, which is dependent on increased pHi in response to migratory cues. Using H⁺ efflux by the ubiquitously expressed Na-H exchanger NHE1 as a tool to understand pH-dependent cell morphology and cytoskeleton dynamics, we identified the structural basis and functional significance of pH-dependent 1) activity of the GTPase Cdc42 for cell polarity, 2) actin-severing activity by cofilin for de novo actin filament assembly, and 3) talin-actin binding for focal adhesion remodeling. Cdc42 and cofilin activity are regulated by pH-dependent histidine switches for phosphoinositide binding, and talin binding to actin is allosterically regulated by pH. These findings have broad impact on understanding normal epithelial cell biology and pathological processes such as metastatic carcinoma, which is associated with a dysregulated increase in pHi.

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SA6

Molecular imaging of P-glycoprotein activity at the human and non-human primate blood-brain and blood-placental barriers

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P-glycoprotein (P-gp), encoded by the MDR1 (ABCB1) gene, is a major efflux transporter that significantly affects the tissue

distribution of a large number of drugs and therefore their efficacy and toxicity. Genetic and chemical knock-out studies in rodents indicate that the greatest impact of P-gp on tissue distribution of drugs is at the blood-brain barrier (BBB) and the blood-placenta barrier (BPB). If true, one strategy to increase the CNS and fetal delivery of drugs is to chemically inhibit P-glycoprotein. Prior to pursuing this strategy, a few key questions need to be addressed. First, is P-gp at the human BBB and BPB as important as that in rodents in preventing distribution of drugs into the brain and the fetus? Second, if it is, can it be significantly inhibited to increase the delivery of drugs to these privileged compartments? Studies in my laboratory and those of others have begun to address these questions in both humans (BBB) and non-human primates (BPB) by using the non-invasive state-of-the-art technique, positron emission tomography (PET). To do so, we have utilized ¹¹C-verapamil as a prototypic P-gp substrate and cyclosporine A as the prototypic P-gp inhibitor. My presentation will review these studies.

Sasongko et al., *Clin Pharmacol Ther.* 2005 77:503-14.

Hsiao et al., *J Pharmacol Exp Ther* 2006 317:704-710.

Hsiao et al., *Drug Metab. Disp.* 2006;317(2):704-710

Eyal et al., *J. Nucl. Med.* 2009;50(5):798-806.

Supported by NIH AG031485 MH63641, GM32165, P50 HD44404 and U10HD047892

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SA7

Disorders of neutral amino acid transport

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Disorders of amino acid transport have been very influential in the elucidation of amino acid absorption in the kidney and intestine. Molecular identification of the transport systems mutated in these disorders highlights the complexity of amino acid transport and demonstrates its links to more complex pathologies such as blood pressure regulation and epithelial cell differentiation.

Hartnup disorder is an autosomal recessive disorder. It is caused by mutations in the general neutral amino acid transporter SLC6A19. The transporter requires one of the two auxiliary proteins collectrin or ACE2 for surface expression. Collectrin is predominantly found in the kidney, whereas ACE2 is found predominantly in the intestine.

Iminoglycinuria (IG) was first described fifty years ago as an autosomal recessive abnormality of renal transport of glycine and of the imino acids, proline and hydroxyproline. The disorder is caused by mutations in the common proline and glycine transporter PAT2 (SLC36A2). Hyperglycinuria (HG) is frequently observed in heterozygotes. In some individuals SLC36A2 mutants retained residual transport activity; in those cases the urinary phenotypes were modified by additional mutations in the proline transporter IMINO (SLC6A20).

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SA8

Aquaporin water channels: from atomic structure to malaria

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The aquaporin water channel proteins confer high water permeability to biological membranes. Discovered in human red cells, AQP1 has been thoroughly characterized biophysically, and the atomic structure of AQP1 has been elucidated. Twelve homologous proteins have been identified in humans. These are selectively permeated by water (aquaporins) or water plus glycerol (aquaglyceroporins). The sites of expression predict the clinical phenotypes. Individuals lacking Colton blood group antigens have mutations in the AQP1 gene. When deprived of water, AQP1-null individuals exhibit a defect in urine concentration and a marked reduction in fluid exchange from lung capillaries. AQP1 is expressed in multiple tissues where physiologically important fluid secretion is known to occur including cerebrospinal fluid in brain and aqueous humor in eye. AQP2 is expressed in renal collecting duct principal cells where membrane trafficking is regulated by vasopressin. Mutations in the human AQP2 gene result in nephrogenic diabetes insipidus, but too little AQP2 expression is found in clinical disorders of urinary concentration, such as lithium therapy and bed wetting. Too much AQP2 expression is found in disorders of fluid retention, such as congestive heart failure and pregnancy. AQP0 is expressed in lens fiber cells and mutations result in familial cataracts. AQP5 is expressed in the apical membranes of salivary and lacrimal glands and sweat glands, and mistargeting may occur in some patients with Sjogren's syndrome. Aquaporins have been implicated in other human clinical disorders such as brain edema, epilepsy, neuromyelitis optica (AQP4), anhidrosis (AQP5) renal tubular acidosis (AQP6). Aquaglyceroporins have been implicated in malaria, skin hydration (AQP3), glucose homeostasis during starvation and protection against arsenic poisoning (AQP7 and AQP9). Aquaporins are known to protect micro-organisms from freezing and osmotic shock. Plant aquaporins are involved in numerous processes including the uptake of water by rootlets and carbon dioxide by leaves. The physiological roles of aquaporin homologs are being pursued by multiple laboratories worldwide.

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SA9

Intestinal sugar absorption via GLUT2: from mice to humans

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GLUT2 is a facilitative glucose transporter in the liver, pancreas, intestine, kidney and brain. Because it is a low-affinity and high-capacity transporter, GLUT2 transports dietary sugars like glucose, fructose and galactose in a wide range of physiological

concentrations to ensure large bidirectional fluxes of sugars in and out cells. In the intestine, GLUT2 is a major contributor to sugar efflux from the cell through the basolateral side of enterocytes into the blood stream. In the past decade, the understanding of the role of GLUT2 has been improved and this facilitative transporter can now be considered a key element for the control of sugar absorption in the course of a meal. The acute regulation of the location of GLUT2 in enterocytes relies on the presence of sufficiently high concentrations of monosaccharides in the lumen of the small intestine to trigger, within minutes, the translocation of GLUT2 into the apical membrane. This high capacity pathway of sugar transport through enterocytes provides a major route of absorption when dietary sugars are abundant; it has been described in species from insects to mammals. The translocation of GLUT2 is also a target for nutrient sensing mechanisms, among which one can distinguish intestinal sweet taste receptor activation by natural and artificial sweeteners, and hormone signalling especially insulin and GLP-2. Inhibition of sugar absorption by insulin release from the pancreas may be important in the control of glucose homeostasis in the postprandial state and indeed insulin internalizes GLUT2 from the apical membrane back into intracellular vesicles.

The contribution of GLUT2 to metabolic diseases previously appeared modest. However, permanent apical GLUT2, increasing the absorption capacity for sugar, is found in experimental diabetes and insulin resistance induced by an excess of sugar and fat in the diet. In obese patients, we found GLUT2 located in the apical membrane of enterocytes even after an overnight fast. Thus the regulation of apical and basolateral location of GLUT2 is profoundly altered in these patients and this might be an issue in the context of overweight and metabolic management of obesity.

Kellett G L, Brot-Laroche E, Mace O J, Leturque A. Sugar absorption in the intestine: the role of GLUT2. (2008) *Annu Rev Nutr*; 28:35-54

This work as supported by INSERM, UPMC P6 University and CNRS

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SA10

Epithelial zinc transport and its regulation in health and disease

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The fundamental importance of both cellular and systemic zinc homeostasis, achieved in part through the regulation of transmembrane zinc flux, is highlighted by the prevalence of human genes coding for zinc-containing proteins, which may comprise as many as 10% of all coding sequences. The multiple human transmembrane zinc transporters are classified as families SLC30 and SLC39. Members of the SLC39 family appear to be involved predominantly in cellular zinc efflux or intracellular

sequestration in membrane-bound compartments, whereas transporters of the SLC39 family have, largely, a role in zinc transport in the opposite direction. Emerging evidence indicates that at least some zinc transporters can function bidirectionally (e.g. (Valentine et al., 2007)). Regulation of epithelial zinc transport, consistent with systemic and/or cellular zinc homeostasis, is achieved, at least in part, through the regulated expression of zinc transporter genes, including at the level of transcription and through RNA stability. As an example, human intestinal zinc transporter expression was seen to be affected by daily oral zinc supplementation (Cragg et al., 2005), and numerous reports document changes in zinc transporter expression in response to changes in zinc availability in rodent and cell line models. Mechanisms of transcriptional regulation in response to zinc in mammalian cells, other than the well-characterised positive transcriptional response to zinc mediated by the transcription factor MTF1, are still to be uncovered. Transcriptional regulation of the SLC30A5 gene, which codes for the zinc transporter ZnT5, localised apically in the enterocyte, appears to provide a model system for the identification of a novel zinc-sensitive transcriptional regulatory pathway (Jackson et al., 2007); ongoing studies based on this response are aimed towards identification of a novel zinc-regulated transcription factor responsible for transcriptional repression at elevated zinc concentrations. Ageing may be associated with changes in epithelial zinc transport, including reduced dietary zinc absorption (Fairweather-Tait et al., 2007). We are currently investigating epigenetic processes as a possible mechanism through which ageing may result in reduced expression of intestinal zinc transporters and, thus, reduce dietary zinc absorption in older people. Abnormal expression of specific zinc transporters has been linked with cancer, including tumours of the breast and prostate (Hogstrand et al., 2009). The SLC30A5 gene is methylated in vivo and promoter methylation in vitro affects expression. In light of the well-established phenomenon that tumour tissue shows changes in DNA methylation compared with corresponding normal tissue, these observations suggest that zinc transporter gene methylation may be a factor in cancer development and/or progression.

Cragg RA, Phillips SR, Piper JM, Varma JS, Campbell FC, Mathers JC & Ford D (2005). Homeostatic regulation of zinc transporters in the human small intestine by dietary zinc supplementation. *Gut* 54, 469-478.

Fairweather-Tait SJ, Harvey LJ & Ford D (2007). Does ageing affect zinc homeostasis and dietary requirements? *Exp Gerontol* 43, 382-388.

Hogstrand C, Kille P, Nicholson RI & Taylor KM (2009). Zinc transporters and cancer: a potential role for ZIP7 as a hub for tyrosine kinase activation. *Trends Mol Med* 15, 101-111.

Jackson KA, Helston RM, McKay JA, O'Neill E D, Mathers JC & Ford D (2007). Splice variants of the human zinc transporter ZnT5 (SLC30A5) are differentially localised and regulated by zinc through transcription and mRNA stability. *J Biol Chem* 282, 10423-10431.

Valentine RA, Jackson KA, Christie GR, Mathers JC, Taylor PM & Ford D (2007). ZnT5 Variant B Is a Bidirectional Zinc Transporter and Mediates Zinc Uptake in Human Intestinal Caco-2 Cells. *J Biol Chem* 282, 14389-14393.

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SA12

Pathophysiology of epithelial Ca²⁺ signalling

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Yoshio Maruyama and I summarized our discovery and characterization of Ca²⁺-activated ion channels in epithelial cells in a review article published 25 years ago (Petersen and Maruyama, 1984). Ca²⁺- and voltage-activated high-conductance K⁺ channels as well as Ca²⁺-activated non-selective monovalent cation channels were located in the baso-lateral membranes of exocrine acinar cells, whereas Ca²⁺-activated Cl⁻ channels were proposed to be located in the apical (luminal) membrane. We only proved the specific Cl⁻ channel localization many years later (Park et al. 2001). In recent years, high-resolution confocal and two-photon studies of the dynamics of [Ca²⁺] in the cytosol, all major organelles and the immediate extracellular environment of pancreatic acinar cells have provided a detailed understanding of physiological (Petersen & Tepikin, 2008) and pathological (Petersen et al, 2009) Ca²⁺ signalling processes. Studies on pancreatic acinar cells have shown that intracellular trypsin activation, which initiates the often fatal human disease acute pancreatitis, is initiated by global sustained elevations of the cytosolic Ca²⁺ concentration (Petersen et al, 2009). Recent data from my laboratory, involving studies on pancreatic acinar cells isolated from IP₃ receptor knock-out mice, have shown directly that trypsin activation elicited by non-oxidative alcohol metabolites depends specifically on excessive Ca²⁺ release via acid store IP₃ receptors of types 2 and 3 (Gerasimenko et al. 2009). This study has for the first time identified the mechanism of alcohol-related acute pancreatitis.

Gerasimenko JV, Lur G, Sherwood MW, Ebisui E, Tepikin AV, Mikoshiba K, Gerasimenko OV and Petersen OH. Pancreatic protease activation by alcohol metabolite depends on Ca²⁺ release via acid store IP₃ receptors. *Proc. Natl. Acad. Sci. USA* 2009; 106: 10758-10763.

Park, MK, Lomax, RB, Tepikin AV and Petersen OH. Local uncaging of caged Ca²⁺ reveals distribution of Ca²⁺-activated Cl⁻ channels in pancreatic acinar cells. *Proc. Natl. Acad. Sci. USA* 2001; 98: 10948-10953.

Petersen OH and Maruyama Y. Calcium-activated potassium channels and their role in secretion. *Nature* 1984; 307: 693-696.

Petersen OH and Tepikin AV. Polarized calcium signalling in exocrine gland cells. *Annu. Rev. Physiol.* 2008; 70: 273-299.

Petersen OH, Tepikin AV, Gerasimenko JV, Gerasimenko OV, Sutton R and Criddle DN. Fatty acids, alcohol and fatty acid ethylesters: toxic Ca²⁺ signal generation and pancreatitis. *Cell Calcium* 2009; 45: 634-642.

Ole H. Petersen is an MRC Research Professor

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permselective properties are quite variable among epithelia. Variations are observed in electrical resistance, ionic charge selectivity and the size-dependence of solute permeability. These properties are the passive complement to active transcellular transport in determining overall transepithelial movement of ions and solutes. There are over 40 different tight junction proteins and consensus is emerging about the contribution of some of these to defining barrier properties (Van Itallie & Anderson, 2006). The continuous cell-cell adhesive seal is formed by members of the large family of claudin transmembrane proteins. Claudins also determine ionic charge selectivity through electrostatic effects of their extracellular protein sequences. Permeability depends strongly on solute size and can be modeled as having two components. Solutes which are smaller than ~4 Å in radius pass through a higher capacity system of charge-selective pores which are presumably formed by claudins. Permeability for larger solutes is independent of ionic charge and size and speculated to follow breaks in the cell-cell contacts (Van Itallie et al., 2008). The magnitude of the second pathway is controlled by cytoplasmic coupling to the scaffolding proteins like ZO-1 and the perijunctional actin-myosin cytoskeleton. This presentation will review current ideas about how selectivity of paracellular transport is defined and regulated by specific tight junction proteins and signaling pathways. Anderson, J.M. and C.M. Van Itallie. 2008. Quick Guide on Tight Junctions. *Current Biology*. 18(20):R941-3.

Van Itallie, C.M. and J.M. Anderson. 2006. Claudins and epithelial paracellular transport. *Annual Review of Physiology*. ed. G. Giebisch, Annual Reviews, Palo Alto CA 68:1-27.

Van Itallie, C.M., Holmes, H., Bridges, A., Gookin, J., Coccaro, M., Proctor, W., Colegio, O. and J.M. Anderson. 2008. The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *J Cell Sci* 121(Pt 3):298-305.

This work was funded by the National Institutes of Health, USA.

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SA14

Creating selectivity in paracellular transport

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Tight junctions create the major barrier to paracellular transport across epithelia (Anderson & Van Itallie, 2008) and their