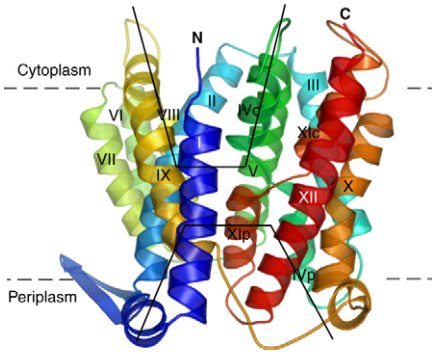
Inside JEB highlights the key developments in *The Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

SOLUTE TRANSPORTERS AND ACID–BASE REGULATION



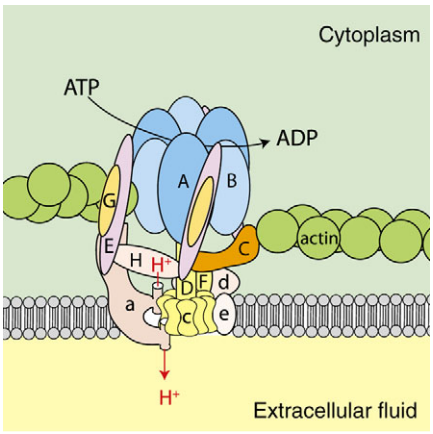
The physiology of acid–base regulation and epithelial transport are core subjects for *The Journal of Experimental Biology*. Over the years the journal has published several reviews on the subject, but with recent advances in our understanding of the molecular basis of acid–base regulation and the development of new model systems, the Editors at *The Journal of Experimental Biology* felt that it was time to revisit the topic with a new collection of review articles primed for the second decade of the 21st century. Covering topics ranging from the structures of the Na^+/H^+ antiporter and acid pumping V-ATPase to the mechanisms of acid–base regulation in tissues such as the *Drosophila* midgut and the mammalian epididymis, the collection covers subjects from both the comparative and biomedical realms, offering a truly integrated perspective on our understanding of acid–base regulation in 2009.

The issue is opened by Etana Padan's description of the three dimensional structure of NhaA, the Na^+/H^+ antiporter from *E. coli*. Outlining the antiporter's role in cellular pH regulation and sodium homeostasis, Padan and her colleagues also point out that the protein is regulated by pH. Focusing on the crystal structure, the team describes how the active monomer is composed of 12 transmembrane helices surrounding cytoplasmic and periplasmic funnels, which are separated by a hydrophobic barrier. They predict that sodium ions bind near the pit of the cytoplasmic funnel and that the pH sensor, situated at the mouth of the cytoplasmic funnel, could activate the antiporter by inducing a conformational change in two of the transmembrane helices to remove the hydrophobic barrier and permit Na^+/H^+ exchange. Pointing out that Na^+/H^+ antiporters play pivotal roles in human diseases such as heart hypertrophy, ischaemia and reperfusion, Padan and her colleagues have modelled the structure of human NHE1, based on the NhaA crystal structure, 'representing an important step towards structure-based drug design,' says Padan (p. 1593).

THE STRUCTURE AND FUNCTION OF V-ATPases

Continuing with the structural theme, Nathan Nelson and Shai Saroussi describe what is known about the structure and function of the proton pumping V-ATPase. Outlining the protein's role in a wide range of organelles, including vacuoles, lysosomes, synaptic vesicles and the Golgi apparatus, the pair discuss the origins of and the structural similarities between the ubiquitous V-ATPase and the ATP producing F-ATPase. While there are many similarities between the two proteins, Nelson and Saroussi point out a fundamental difference between the composition of the proteins' c-rings, with the F-ATPase c-ring comprising 10–14 identical units while the V-ATPase c-ring is composed of three different subunits. The electron transporting subunit, a, is also much larger in the V-ATPase than the F-ATPase. Discussing the possible mechanism of ATP hydrolysis, which drives electron transport across the membrane, the duo raise some concerns that the widely accepted rotary mechanism may not be correct. Finally, Nelson and Saroussi outline the 3-D structures of several V-ATPase subunits, including the c, H, F and B subunits (p. 1604).

Following on from Nelson and Saroussi's discussion of V-ATPase structure, Helmut Wieczorek, Klaus Beyenbach, Markus Huss and Olga Vitavska discuss the role of the V-ATPase in active transepithelial transport of alkali metal ions in insects. They outline how V-ATPases transport H^+ across a membrane establishing a transmembrane voltage that drives an antiporter, which, depending on the antiporter, exchanges H^+ for either K^+ or Na^+ ions. Focusing first on the V-ATPase in the tobacco hornworm, Wieczorek and his colleagues discuss what is known about the protein's structure, before discussing what is known about the interactions of inhibitors with the V-ATPase. The team also explains how the V-ATPase is reversibly inactivated in the digestive tract during moulting and starvation. This down regulation makes sense because nutrient uptake consumes large amounts of ATP, and so it is stopped under these conditions. V-ATPases also play a critical role in fluid secretion in the *Drosophila* and *Aedes aegypti* Malpighian tubules (analogous to the vertebrate kidney), and Wieczorek and colleagues conclude by laying out some of the challenges that lie ahead, such as discovering the regulatory mechanisms for the ATPase and the role of V-ATPase



interactions with other cell structures such as the cytoskeletal scaffold (p. 1611).

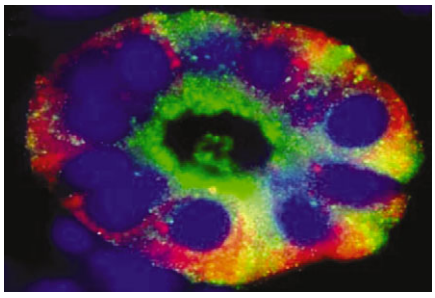
Completing the discussion of the role of V-ATPases in ion transport, William Harvey, from the University of Florida, discusses Peter Mitchell's chemiosmotic hypothesis for energising membranes for ATP synthesis. Harvey explains that the theory has been applied to membrane energization by V-ATPases, and outlines his alternative membrane energisation theory, based on Douglas Kell's expansion of Mitchell's model. Harvey suggests that instead of acidifying the bulk solution surrounding a membrane, the V-ATPase charges the membrane in such a way that it behaves like a capacitor, imposing a voltage across the membrane which is then used to power transporters in the membrane. Harvey goes on to present examples of transepithelial transport, such as the caterpillar K^+ pump (formed by a V-ATPase and $K^+/2H^+$ antiporter) and Na^+- or K^+-coupled nutrient amino acid transporters, and applies the new model to explain each system's transport properties (p. 1620).

CATION EXCHANGERS IN ACID–BASE BALANCE

Having outlined the role of the V-ATPase in establishing a pH gradient and electromotive force to drive secondary transport, the collection of reviews shifts to the mechanisms that underlie cation exchange in acid–base regulation. Todd Alexander and Sergio Grinstein from the Hospital for Sick Children in Toronto, Canada, focus on one of the nine known mammalian Na^+/H^+ exchangers, NHE3, which is critical for intravascular volume control, salt reabsorption and pH regulation, and is strongly regulated by digestion (p. 1630). Describing how the exchanger is located largely in the kidney and gastrointestinal tissue epithelial cells, Alexander and Grinstein discuss the function of the exchanger where Na^+/H^+ exchange is modulated by H^+ to reduce exchange and prevent excessive alkalinisation. The duo also list the hormones that are known to modulate the exchanger's activity, including parathyroid hormone and dopamine, which inhibit the exchanger, and angiotensin II and the glucocorticoids, which activate the exchanger.

Mark Donowitz and his colleagues from Johns Hopkins continue the discussion of cation exchangers with their review of the regulatory complexes that associate with the C-terminal domain of mammalian NHE3 (p. 1638). After presenting the transport processes that NHE3 is involved in, Donowitz focuses on the functions of the protein's N-terminal Na^+/H^+ transporter domain, and the regulatory C-terminal domain. Donowitz explains that the transport domain is thought to be composed of 11 transmembrane helices, unlike the 12 found in the *E. coli* Na^+/H^+ exchanger, and that amino acids critical for transport of both ions in the *E. coli* transporter are also found in NHE3. Moving to the C-terminal domain, Donowitz explains that the domain is essential for the regulation of Na^+/H^+ transport. According to Donowitz, the C-terminal domain fullfills two functions; serving as a scaffold linking the protein to the cytoskeleton while distinct regions of the domain also regulate specific aspects of the NHE3 transport activity. Donowitz suspects that the large number of proteins associated with the C-terminal domain may regulate the protein by changing their associations with NHE3 and each other, and he hopes that studies of the proteins that associate with a small helical region, an area he has called the switch domain, 'will provide insights into the defining aspects of NHE3 function in intestinal Na absorption'.

CARBONIC ANHYDRASE IN ACID–BASE BALANCE



Carbonic anhydrase (CA) is a key enzyme in acid–base regulation, involved in CO_2 transport by the reversible hydration of CO_2 to HCO_3^-. Katie Gilmour and Steve Perry from the University of Ottawa give an overview of the large number of CA isoforms identified in fish (p. 1647). According to Gilmour and Perry, fish cannot manage their acid–base balance by 'blowing off' CO_2 to increase pH. They have to differentially regulate the efflux of HCO_3^- and H^+ by increasing excretion of both across the gill during acidosis and varying their response depending on whether the acidosis is caused by respiration or metabolic effects. After listing the CA isoforms and their cellular locations in teleost, elasmobranch and agnathan fish gills, Gilmour and Perry go on to look at the role of CA in the kidney. Finally, Gilmour and Perry outline the role of CA in other fish tissues, such as the elasmobranch rectal gland, swim bladder and pseudobranch, and conclude that 'the diversity of CA isoforms and functions in fish is at least as great as that in tetrapods, if not more so,' yet our knowledge of CA isoforms 'lags well behind that for mammals,' and must be addressed if we are to completely understand the role of CA in acid–base regulation in fish.

Moving on from CA in fish acid–base regulation, Paul Linser and colleagues from The University of Florida Whitney Laboratory discuss the enzyme's role in mosquito midgut pH regulation. The anterior portion of the larval mosquito midgut is one of the most alkaline environments known. Given the mosquitoes' pivotal role as a disease vector, there has been much interest in controlling the insect population by interfering with larval digestion, requiring an understanding of the role of CA in pH regulation in the larvae's gut. With the *Anopheles gambiae* genome in hand, Linser describes how it has been possible to identify 12 CA genes in the insect's genome, and to identify their distribution in the insect's midgut as a source of carbonate and bicarbonate (p. 1662). Linser and his colleagues highlight two CAs, AgCA9 and AgCA10, which are probably central to maintaining the pH in the larvae's gut, before going on to explain how the enzyme also plays a role in bicarbonate and carbonate excretion through the hindgut.

ANION EXCHANGERS IN ACID–BASE BALANCE

Intracellular pH is also regulated by a group of Cl^-/HCO_3^- exchangers encoded by two gene superfamilies: *SLC4* and *SLC26*. Focusing on the SLC4 anion exchangers (p. 1672), Seth Alper from Harvard Medical School describes the current understanding of the structure of SLC4A1/AE1, and the monovalent anions that are electroneutrally exchanged by the protein. Although the mechanism of anion selectivity is not currently known, Alper goes on to discuss the inherited disorders, including red blood cell disorders and distal renal acidosis, which result from loss or mutations of the SLC4A1/AE1 anion exchanger. Alper also

outlines diseases of other tissues associated with mutations in the *SLC4A2/AE2* and *SLC4A3/AE3* genes. Commenting on regulation of the SLC4 proteins by pH, Alper points out that despite functioning in the sometimes-extreme pHs found in the renal medulla, AE1 is regulated only modestly by pH, although AE2 function is highly pH sensitive.

Following Alper's discussion of SLC4 Cl^-/HCO_3^- exchangers, Martin Grosell and colleagues from the University of Miami discuss Cl^- absorption and HCO_3^- secretion in the intestines of marine teleosts, resulting in $CaCO_3$ precipitates in the fish's intestines (p. 1684). Grosell describes proteins that may be involved in Cl^-/HCO_3^- exchange before moving on to discuss the origin of the HCO_3^- secreted into the intestine and the mechanisms for removal of H^+, which results from the hydration of CO_2 by CA. This led to the unexpected discovery of a H^+ pump in the apical membrane of the intestine epithelium. The team then go on to show that SLC26a6 Cl^-/HCO_3^- exchanger is coupled with an apical V-ATPase, which together form a functional unit that Grosell and his colleagues refer to as a 'transport metabolon', which is responsible for the electrochemical uphill absorption of Cl^- and HCO_3^- secretion into the intestine.

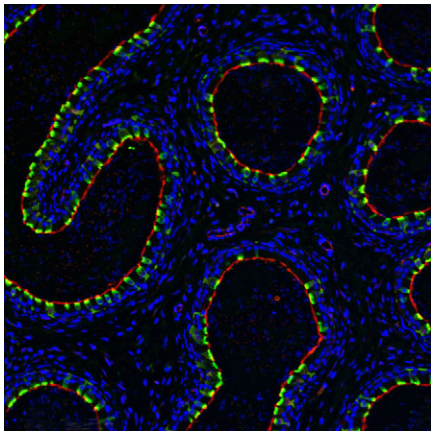
TRANSPORTERS, EXCHANGERS AND CHANNELS

Another class of proteins that are inextricably linked with cellular pH regulation are the Na^+ coupled bicarbonate transporters (NCBTs), which mediate the coupled movement of Na^+ and HCO_3^-. Walter Boron and his colleagues from Case Western Reserve University Medical School outline the tissues where each of the NCBTs are expressed and the genes which encode each protein, before going on to discuss the protein motifs that comprise these structurally diverse proteins (p. 1697). The team presents an alignment of over 21 NCBT sequences and discusses the sequence motifs found in the long N-terminal region and the proteins that these regions are known to interact with. Next the team discuss the highly conserved NCBT transmembrane domain, suggesting that subtle sequence differences between the proteins are responsible for the different ratios of Na^+ and HCO_3^- transported by each NCBT. Having finally listed the structural properties of the NCBT C-terminal regions, which vary significantly between proteins, Boron and his team conclude by saying, 'Our increasing knowledge of the diverse roles of NCBTs and their variants is shedding light on the necessity of their seemingly bewildering structural diversity.'

Switching from electrogenic transporters to the largely water transporting aquaporins, Alan Verkman from the University of California summarises the roles of five aquaporins (AQP) in water transport across kidney tubules. Verkman also discusses the roles of AQP4 in the removal of excess fluid from the brain in vasogenic brain oedema and neural function, and AQP1 in cell migration (p. 1707). According to Verkman, AQP3 and AQP7 function additionally in glycerol transport. AQP3 has also been implicated in cell proliferation, as mice lacking the protein are unable to develop skin tumours, 'AQP3 inhibitors may thus have utility in skin tumour prevention and therapy,' says Verkman. Given the mounting evidence of the role of various aquaporins in a variety of diseases, Verkman lists scenarios where aquaporin inhibitors may provide viable therapies, although none are currently available. He also describes how a serum immunoglobulin that targets AQP4 epitopes has been shown to be diagnostic of a rare form of multiple sclerosis, Devic's disease. Finally, Verkman closes by discussing rare disorders caused by loss-of-function mutations in human aquaporins, and the use of aquaporin polymorphisms as disease markers.

Having considered water transport, the collection of reviews moves on to the topic of nitrogenous waste excretion in the form of ammonia and urea transport across the gills of fish and aquatic crustaceans. Dirk Weihrauch, Michael Wilkie and Patrick Walsh outline the mechanisms of ammonia and urea transport in adult freshwater teleosts, the embryonic and larval life stages of aquatic organisms and saltwater teleosts (p. 1716). They introduce the Rh (Rhesus) proteins as members of the Amt/MEP/Rh family of proteins, which have been shown to transport ammonia and are expressed in fish gills. Although the role of Rh proteins in ammonia transport is still unclear, the team say, 'it is likely that some form of Rh-mediated passage of NH3 occurs'. Weihrauch and colleagues also discuss the problems faced by amphibious fish, which must either convert ammonia to urea or excrete ammonia directly into the air, and suggest that Rh proteins are involved in ammonia excretion in slender Africa lungfish, giant mudskippers and the mangrove killifish. The team also suggest that 'primitive' fish, such as hagfish and lampreys, both of which experience high ammonia loads, could teach us much about the genetics and function of Rh proteins, given the fish's early emergence on the evolutionary tree. Weihrauch concludes the review with a warning that although it is clear that Rh proteins are involved in nitrogenous waste excretion to some extent, 'we must be rather cautious in our rush to embrace Rh proteins as the *sine quo non* of ammonia transport in aquatic species.'

INTEGRATING GENES AND PHYSIOLOGICAL FUNCTION



Having reviewed the roles of a suite of proteins involved in acid–base regulation and transport, the collection moves on to discuss the physiological function of many of these proteins in four unique systems. Turning first to the *Drosophila* midgut, Shubha Shanbhag and Subrata Tripathi from the Tata Institute of Fundamental research in India explain why the recent identification of stem cells in the midgut has made physiological characterisation of cellular transport in the tissue essential; '*Drosophila* midgut epithelial development and maintenance is analogous to mammalian intestinal crypt development' they explain (p. 1731). Focusing on both the larval and adult midguts, Shanbhag and Tripathi describe both life stages' lumen pH profiles, the epithelial cellular structures and what is currently known about the transport processes that operate in individual midgut sections.

Moving on to another model organism, Pung-Pung Hwang discusses the molecular and cellular characterisation of ion regulation in zebra fish (p. 1745). Listing the three cell types (H^+-ATPase rich cells, Na^+–K^+-ATPase rich cells and Na^+–Cl^- cotransporter cells) involved in ion uptake and acid–base regulation in zebrafish skin and gills, Hwang outlines the V-ATPases, transporters, enzymes and the pathways in which they function in transport in each cell type. Hwang concludes with the observation that the zebrafish is proving to be a powerful *in vivo* working model for mammalian renal transport given the wealth of genomic data and new methodologies available in the model organism.

Another system that requires fine acid–base regulation is the male reproductive system, where spermatozoa are stored and remain dormant at acidic pH until activated by a cascade of events including an influx of bicarbonate. In the review by Winnie Shum, Nicolas Da Silva, Dennis Brown and Sylvie Breton, the team from Harvard Medical School discuss V-ATPase regulation in the apical membrane of epididymis clear cells, which contribute significantly to acidification of the epididymis lumen (p. 1753). The team explain how accumulation of V-ATPase in the clear cell apical membrane is regulated by recycling of the pump between vesicles and the apical membrane, which is modulated by actin depolymerization and signals from other cells in the epithelium, to achieve 'fine control of an optimum acidic luminal environment that is critical for male fertility,' says Breton and her collaborators.

Acid–base regulation is also essential in the production of urine, and the kidney is another tissue where proton excretion by V-ATPases is essential. Dennis Brown, Teodor Paunescu, Sylvie Breton and Vladimir Marshansky discuss the role of V-ATPase in two types of cell, known as intercalated cells, found in the late distal tubule, the connecting segment and the collecting duct of the kidney (p. 1762). Explaining that the A form of the intercalated cell is responsible for acid secretion into the tubule lumen, while the B form secretes bicarbonate, the team outlines the distribution of the individual molecular components for acid and bicarbonate excretion in the two cell types. They also discuss how incorporation of the B1 isoform in the intercalated cell V-ATPase appears to produce a stronger proton pump than the B2 subunit and suggest that the V-ATPase proton transport activity in intercalated cells may be regulated by soluble adenylate cyclase in response to HCO_3^-.

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