the University of California in San Diego where, in addition to maintaining their research programmes, George became Dean of Scientific Affairs at the School of Medicine.

Concluding remarks

At a recent one-day symposium that was held in his honour in La Jolla (FIG. 5), George sounded like the son of the professor of philosophy that he was. He reminded us that, although we have come far since the 1940s, we still have very far to travel. Our collective intellectual itinerary cannot be charted in advance, and will be enriched beyond all expectation as more approaches and experimental systems become part of our armamentarium. Those who identify with George's enduring commitment to an integrative view of the dynamics of organelles were especially moved when he showed a list of former colleagues, and said that it "…reads like a poem." Regardless of whether one has worked directly with him or not, it has always been a great privilege to be part of George's ensemble.

Alan M. Tartakoff is at the Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, USA.
e-mail: amt10@po.cwru.edu

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Alan M. Tartakoff's lab:
http://www.cwru.edu/med/pathology/fac/tartakoff.htm
George E. Palade's Nobel lecture:
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OPINION

The significance of molecular slips in transport systems

Nathan Nelson, Ayelet Sacher and Hannah Nelson

The advantage of precision in biological processes is obvious; however, in many cases, deviations from the faithful mechanisms occur. Here, we discuss how in-built operating imperfections in transport systems can actually benefit a cell.

Without the genetic property of slight blundering, the evolution of advanced life forms would be impossible — random mutations, as well as imperfect DNA replication and repair, are essential to life[1]. Indeed, imperfections are necessary for the adaptation and smoothness of operation in many biological processes[2–4].

Certain biochemical processes function by coupling extremely precise reactions with others that enjoy a high degree of freedom. Take photosynthesis, for example. The structure of the reaction centres that harvest light and convert its energy into electrochemical energy is very rigid, and this allows only a small degree of freedom[5]. On the other hand, the electrochemical gradient of protons ($\Delta\mu_{H+}$), which is formed across the thylakoid membrane by the reaction centres, is prone to losses through 'leaks' and 'slips' (BOX 1). Therefore, the ratio of ATP synthesized to electrons transported does not give a fixed stoichiometry, and the combination of a rigid photochemical reaction with biochemical reactions that are prone to leaks and slips has evolved to optimize photosynthesis. Similar arrangements of coupling and slippage mechanisms operate in the respiratory chain[6,7].

Here, we discuss the possibility that a slip mechanism not only exists in complex systems, such as nucleic-acid replication, photosynthesis and respiration, but also modulates the function of transport systems. This slippage in the driving force and/or in the transport pathway might provide a safety valve through which an excess driving force or substrate is dissipated. We propose that slips are fundamental for life processes, as they fulfil the cell's need to deal with stressful situations that are created by transitory extreme conditions, which might otherwise interfere with the proper function of a cell and even jeopardize its existence. To illustrate this point, we discuss proton ATPases and the ion- or substrate-translocation pathways of ion-motive transporters as examples of proteins that might have adapted such in-built molecular slips.

Ion-motive ATPases

Evolutionarily, ion-motive ATPases can be grouped into two distinct families: the P-ATPases, which operate by means of a phospho-enzyme intermediate, and the F- and V-ATPases, which operate by means of mechanochemical movements[8]. However, all of these ATP-dependent ion pumps couple a scalar reaction of ATP hydrolysis (or

"…imperfections are necessary for the adaptation and smoothness of operation in many biological processes."

Box 1 | Leaks and slips

The terms 'leaks' and 'slips' have been used to describe an observed deviation from the expected stoichiometry of an operating system[6,7,69,70]. In this article, we use the term 'leak' whenever a general property of the membrane is addressed — that is, we use the term to describe the summation of the membrane's permeability. We use the term 'molecular slip' when the property of an operating complex or individual protein is concerned — that is, a molecular slip is an in-built feature of a specific protein or complex.

synthesis) to a vectorial movement of ions across membranes.

The general structures of the F- and V-ATPases are quite similar (FIG. 1), although only the F-ATPase can efficiently synthesize ATP[9–11]. Both enzymes are composed of numerous subunits that are clustered into catalytic and membrane sectors. The main function of the catalytic sector is to catalyse ATP synthesis or ATP hydrolysis, whereas the main function of the membrane sector is to conduct protons across the membrane. The coupling of these two sectors and processes occurs through mechanochemically induced conformational changes[12,13].

F-ATPase. The F-ATPase is a multisubunit mechano-enzyme that produces ATP at the expense of ion (H^+)-motive force, or vice versa[9,14]. FIGURE 1a depicts a schematic representation of the subunit composition of F-ATPase. It was generally assumed that ATP-dependent ion pumps such as the F-ATPase are tightly coupled, and that loose coupling, when observed, can be attributed to extrinsic leaks. In a recent and elegant experiment, ATP-dependent rotation of an artificial rod that was attached to the γ-subunit of the catalytic (F_1) part of the enzyme was measured, and the results indicated that there was 100% coupling between ATP hydrolysis and the mechanistic torque[15,16]. However, this observation related to a very small proportion of the enzyme population, and the authors could not measure the ATPase activity. Moreover, as the membrane-bound (F_0) part of the enzyme was absent in this system, these results cannot be related to the coupling of ATPase activity with proton translocation.

Recent structural studies[13–18] showed that F-ATPase operates with a different H^+/ATP stoichiometry in mitochondria, chloroplasts and bacterial membranes. This could be due to the fact that there are thought to be different numbers of c-subunits in the F-ATPases of the different organisms studied, and perhaps even within the same organism (FIG. 1). Three molecules of ATP are formed or hydrolysed per 360° cycle, and, as each c-subunit carries one proton, the number of c-subunits per complex should determine the stoichiometry[14]. Because the number of c-subunits in different organisms varies from 10 to 14, the H^+:ATP ratio is unlikely to be an integer (except where 12 c-subunits are present)[18–20]. Similary, the coupling between electron transport and ATP formation is also unlikely to be an integer, even if the ratio of electrons:protons transported is an integer.

Over the past 25 years, several reports have shown that a certain degree of intrinsic uncoupling is present in electron-transport systems and ion pumps[6], and that protons slip through the F-ATPase in chloroplasts[21–23]. The evidence for this was that the measurement of electron:ATP ratios in oxidative phosphorylation and photophosphorylation gave different values in various different laboratories[24].

These differences can be attributed to two main factors. The first is the stoichiometry of the proton:ATP ratio at which the specific ATPase operates, and the second is the proton leak that has to be taken into account and subtracted from the coupled reaction in those different experimental systems[24]. It seems that part of the uncoupled proton conductance across the membrane proceeds through F-ATPases under substrate limitations. Indeed, the observed uncoupled currents can be modulated by various F-ATPase substrates and specific inhibitors, which indicates that the protons are conducted through the enzyme[21–23,25]. So, this uncoupled proton conductance is probably due to a proton slip that takes place during certain operational modes of the F-ATPases.

V-ATPases. In contrast to the F-ATPases, whose main function in eukaryotic cells is to form ATP at the expense of proton-motive force (PMF), V-ATPases in eukaryotes function exclusively as ATP-dependent proton pumps[26], and cannot efficiently catalyse PMF-driven ATP synthesis. Nevertheless, we assume that the same basic structure and mechanism of ATP-dependent proton pumping operates in both F- and V-ATPases (FIG. 1).

The inability of V-ATPases to produce ATP at a reasonable rate was attributed to the presence of an in-built proton slip[27]. As shown in FIG. 2, at a PMF of 0 mV across the membrane, V-ATPases operate as a fully coupled, efficient proton pump. The efficiency decreases with the build-up of PMF and, in most cases (for example, in chromaffin granules), at a PMF of about 120 mV the slip is equivalent to the proton pumping and there is no further build-up of PMF. A PMF of 120 mV is below the thermodynamic equilibrium of the system, which precludes the efficient formation of ATP[28,29].

The threshold for proton slippage could occur at a different pH and/or membrane potential value in different systems. For example, whereas *Saccharomyces cerevisiae* vacuoles maintain an internal pH of about 5.5, lemon fruit vacuoles can have a pH as low as 2, yet the vacuolar pH in both organisms is controlled by V-ATPases[30–32]. In our opinion, variation in the extent of the slip in these pumps could explain, at least in part, the different pH values in different organelles[27,32]. We suggest that such an in-built proton slip mechanism is a general feature of the V-ATPases — that is, under back

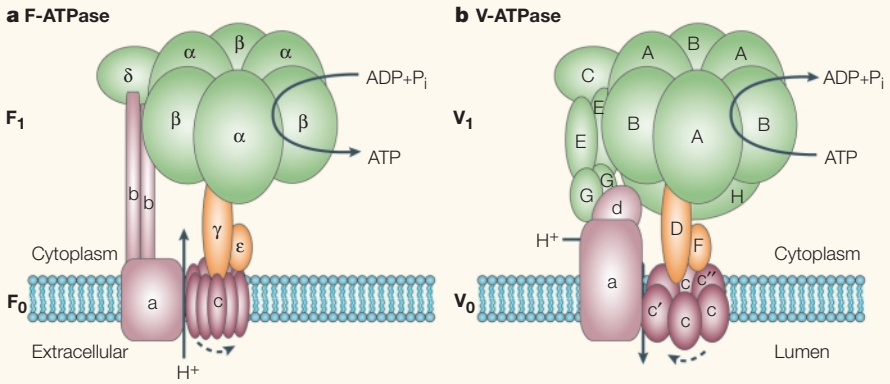

Figure 1 | **Subunit structure of the F- and V-ATPases.** The schemes depict the subunit composition of **a** | the *Escherichia coli* F-ATPase and **b** | the yeast V-ATPase. The subunits of the F_0 and V_0 membrane sectors are shown in pink, and those of the F_1 and V_1 catalytic sectors are shown in green and orange. The c-, γ- and ε-subunits rotate in the F-ATPase, and the c- (c, c′ and c″), D- and F-subunits rotate in the V-ATPase. The dashed arrows indicate the direction of rotation.

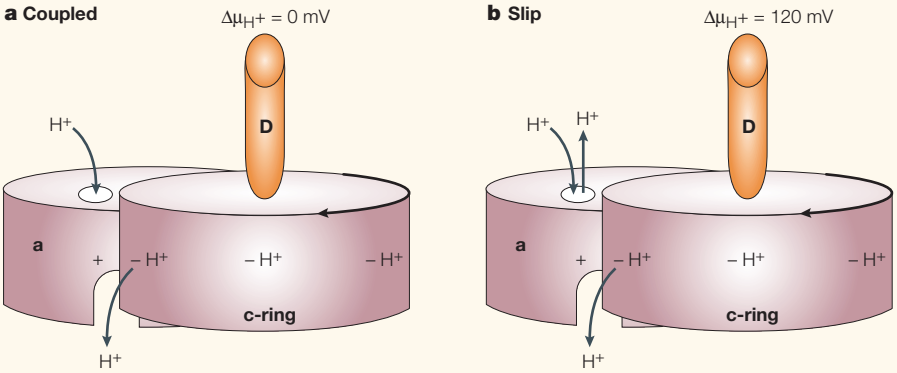

Figure 2 | **A schematic representation of a mechanistic slip induced in V-ATPase by an increased proton-motive force.** The a-subunit and the c-ring are part of the V-ATPase membrane sector that functions as a proton turbine (see FIG. 1 for the subunits that compose the rotating part of the enzyme). **a** | At a proton-motive force (PMF) of 0 mV (when H^+ movement is coupled to ATPase activity), the proton enters the V_0 membrane sector through a specific channel in the a-subunit and binds to a free negative charge on one of the c-ring subunits. The c-ring then turns clockwise until a positive charge on the a-subunit releases it into the lumenal compartment (see FIG. 1). **b** | When the PMF builds up, some of the protons return through the channel in the a-subunit to the cytoplasmic face of the membrane and a proton slip is generated. The D-subunit functions as a shaft that turns the c-ring using the energy from ATP hydrolysis by the catalytic sector (see FIG. 1).

pressure from the proton electrochemical gradient, the complex enables protons to slip back in the opposite direction to the ATP-dependent proton pumping (FIG. 2). The net result is that the PMF cannot exceed a certain value because the slip restricts it to a value below the thermodynamic equilibrium.

Assuming that most of the above considerations are correct, what would the physiological significance of the inherent slip in the mechanism of V-ATPases be? We have to accept the idea that energy conservation is not the main thrust of eukaryotic cells, and that they are willing to pay a cost to maximize the adaptability of physiological processes. So, in several cellular organelles, such as the Golgi apparatus and synaptic granules, prevention of overacidification is more important than energy conservation. This prevention could be achieved by the introduction of a major slip into the mechanism of ATP-dependent proton pumping by V-ATPases. In some cases, however, such as lemon fruit vacuoles or *Ascidia sydneiensis* blood giant cells[30,33], the V-ATPase shows its maximal capabilities — that is, it acts without 'slipping'. An in-built slip mechanism could set a limit for the acidification of organelles by the same enzyme in most eukaryotic vacuolar systems.

Ion-motive transporters

In the past few years, data have accumulated that indicate that it is not only multisubunit complexes, but also single polypeptide transporters, that have incorporated slips to control the coupling between the driving force and the transport process[34–36]. So, the ion:substrate stoichiometry in ion-motive transporters is not a fixed integer and could also vary in accordance with changes in the environment. The variable stoichiometry could again result from the existence of leaks and slips during the transport process (BOX 1). Below, we discuss two examples of transporters that seem to take advantage of slips — neurotransmitter and metal-ion transporters.

Neurotransmitter transporters. For most members of the Na^+/Cl^- neurotransmitter-transporter family[37], the driving force for transport is a sodium electrochemical gradient that is used for the co-transport of two Na^+ ions, one Cl^- ion and one substrate molecule[38]. On the other hand, glutamate transporters — which are Na^+/K^+-dependent transporters — catalyse an electrogenic process in which glutamate is co-transported with three Na^+ ions, followed by the counter-transport of one K^+ ion[39,40].

"…slippage in the driving force and/or in the transport pathway might provide a safety valve through which an excess driving force or substrate is dissipated."

Similar to other proteins that transport substances across membranes, the mechanism of neurotransmitter transporters can be represented by a kinetic scheme[37] . Such a scheme describes partial transport steps, such as ion and substrate binding to specific sites on the external face of the transporter, their translocation across the membrane, their release from the transporter and the return of the transporter to its original conformation. However, uncoupled currents that result from the movement of ions through the transporter, and which are not productive in substrate transport, have been recorded[41–46] in membranes that express Na^+/Cl^--dependent and Na^+/K^+-dependent transporters. This bulk transport does not fit the rigid kinetic schemes, and so it prompted several investigators to describe the mechanism of substrate transport across membranes in terms of channels[35,47]. In particular, it was proposed that substrate binding to the transporter induces conformational changes that favour the formation of channels for ion conductance[43].

Fluctuation analysis of the *Drosophila melanogaster* serotonin transporter, which is an Na^+/Cl^--dependent transporter that can be expressed in *Xenopus laevis* oocytes, has been used to study the existence of channels in this type of transporter. At –20 mV, ~500 serotonin molecules were transported per channel opening[47], and, at the same time, ~10,000 charges were translocated. So, if the formation of channels is responsible for ion conductance, this means that either one channel opening takes place every 500 transport cycles, or 500 serotonin molecules and 10,000 ions pass through a common channel. Both of these suggestions involve major structural alterations in the transporter.

This phenomenon is not unique to Na^+/Cl^--dependent transporters — it has also been observed with Na^+/K^+-dependent transporters expressed in *Xenopus* oocytes. Studies of glutamate transporters have shown that high electrical currents occur in the presence of substrate[48,49]. The anion conductance of the expressed transporters, which is not coupled to their substrate transport activity, showed channel-like properties, with gluconate (a large anion) being more than tenfold less effective in generating currents than the much smaller Cl^- ion[50]. Noise analysis indicated a unitary Cl^- conductance of ~2 femtoamperes (fA) per channel in total[51], and addition of the substrate to the external medium resulted in an electric current of about 100 nA. So, $\sim 5 \times 10^7$ such channels per oocyte are required to generate a current of this magnitude.

However, these properties deviate from those of most conventional ligand-gated

channels, which have much larger pore sizes, and so show much higher conductance values. Ligand-gated channels usually bind their specific agonists, but do not translocate them across the membrane. They operate like gated holes, which enables the movement of ions down an electrochemical gradient or of small molecules down a concentration gradient. By contrast, the main function of ion-motive transporters is substrate uptake, in many instances against the concentration gradient. We propose that it is therefore not necessary to invoke the existence of channels, because the observed uncoupled currents could be explained, more simply, in terms of molecular slips (FIG. 3).

Reports on mammalian and *Drosophila* serotonin transporters that are expressed in *Xenopus* oocytes[41,47], and analyses of these transporters in leech neurons[34], have indicated that the excess movement of charges are intimately linked to the serotonin transport mechanism. The excessive currents were dependent on substrate binding, and even the substrate-independent charge movements in the serotonin transporter were modulated by specific inhibitors of the transporter[41,47,52]. This means that the coupled and uncoupled states are likely to be linked, because similar substrate and ion dependency, as well as antagonist sensitivity, were observed for both.

In *Xenopus* oocytes that were made to express the serotonin transporter at serotonin concentrations above saturation, currents were recorded that resulted from serotonin uptake and not from the movement of inorganic ions[47]. This points to the possibility of a substrate slip. Similarly, an initial transient rapid current (that is, current that results from an abrupt increase of substrate concentration in the synaptic cleft) was observed in leech neurons. This was thought to reflect a synchronous activation of many serotonin transporters, followed by asynchronous operation of the transporters that produced a slow decaying inward current[34]. The recorded currents had the characteristics of channels, which led the authors of this study to suggest that channel formation was the explanation. However, this transient activity could be explained by a configuration that enabled substrate slippage, as depicted in FIG. 3c.

Substitution of Na^+ by Li^+ in the external medium resulted in increased currents in several neurotransmitter transporters that have been expressed in *Xenopus* oocytes[38,41,52–54], which includes the various γ-aminobutyric acid (GABA) transporters (T. Grossman and N.N., unpublished observations). We propose that these uncoupled currents could be generated by a slippage through the driving force pathway of the transporter, as depicted in FIG. 3b.

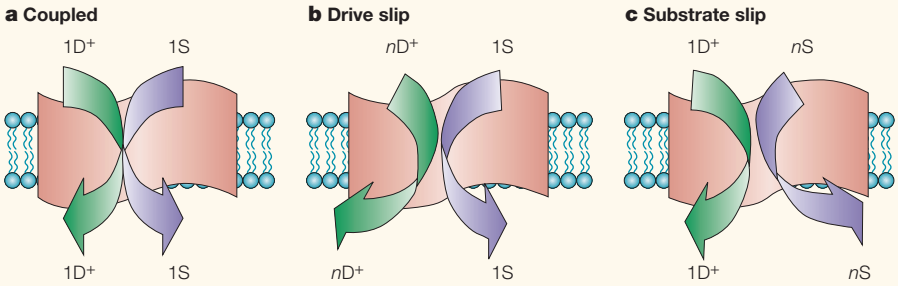

Figure 3 | **A schematic representation of a mechanistic slip in ion-driven transporters.** The wide arrows represent the driving force (D^+) (green) and the substrate (S) (purple) pathways. The variable degree of coupling between the two pathways is depicted by the degree of contact between the two arrows. **a** | In the coupled state, a unit of driving force (ions per substrate molecule) drives the transport of one or more substrate molecules (in accordance with the properties of the transporter). **b** | During 'driving-force slippage', an increased number of ions per substrate molecule cross the driving force pathway. Consequently, the rate of substrate transport is reduced. In the main text, this model is discussed for proton slippage through Dct1, sodium slippage through Smf1, and lithium slippage through neurotransmitter transporters. **c** | The 'substrate slip' is discussed in the main text for serotonin slippage through the serotonin transporter. In this model, the number of transported substrate molecules exceeds the number of ion-driving units that cross the transporter. *n*, any number > 1.

Metal-ion transporters. Transition metals are essential for many metabolic processes, and their homeostasis is crucial for life. Metal-ion transporters provide an efficient tool for the control of metal-ion accumulation and secretion, as well as for defence against bacterial infection[55–63].

The mechanism of metal-ion transport by eukaryotic cells is largely unknown. Most of the information available has come from electrophysiological studies in which the mammalian divalent cation transporter Dct1 — also known as Nramp2 — and the yeast homologue Smf1 were expressed in *Xenopus* oocytes[64–66]. These studies showed that Dct1 transports Fe^{2+} together with H^+. Protons are the driving force for the transport of metal ions by this family of transporters. At pH 7 and membrane potentials of –90 to –30 mV, Dct1 transports one Fe^{2+} ion with one H^+ ion. At a high proton concentration (low pH), the number of H^+ ions transported with one Fe^{2+} ion increased to 10 (REF. 64). Moreover, on changing the membrane potential from +10 to –80 mV at this low pH, the number of H^+ ions transported with one Fe^{2+} ion increased from 3 to ~18 (REF. 65). These results indicate that protons might slip through the driving force pathway (FIG. 3b).

Additional experiments in *Xenopus* oocytes showed further properties of the proton slip mediated by Dct1 (REF. 66). At pH 5.5 and an imposed membrane potential of –50 mV, addition of Co^{2+}, Mn^{2+}, Zn^{2+} or Fe^{2+} generated a current of about 100 nA. The uptake of labelled metal ions was measured under similar conditions, and varied from about 50 pmol oocyte^{-1} h^{-1} for Fe^{2+} to over 100 pmol oocyte^{-1} h^{-1} for Mn^{2+} and Co^{2+}. Remarkably, the oocytes injected with *Dct1* messenger RNA showed no uptake of radiolabelled Zn^{2+}, however, Zn^{2+} generated currents that were as high as the other metal ions[66]. The changes in stoichiometry between the driving force (H^+ translocation) and the substrate (metal ion) uptake indicate that there is an in-built slip in the mechanism of action of this metal-ion transporter. So, the proton slippage in Dct1 is dependent on the presence of metal ions, regardless of whether those particular metal ions are transported across the membrane.

In contrast to Dct1, Smf1 shows no metal-ion-induced proton slippage, even though protons are also the driving force for its metal-ion transport. However, it does show an Na^+ slip, especially at high pH, which is metal-ion independent[65,66]. This slip is observed by recording currents that are generated on the addition of Na^+ to oocytes that express Smf1 (REFS 65,66). A lower pH reduces the Na^+ currents, which indicates that protons compete with Na^+ for a common binding site or translocating pathway. Na^+ also inhibits the transport of metal ions by Smf1 — such an inhibition increases with increasing pH in the medium. When the concentration of protons in the medium decreases (higher pH), Na^+ competes more effectively with H^+ for its binding site, which diminishes the driving force for metal-ion uptake, and generates a large sodium slippage through this route.

What is the mechanism of the H^+ and Na^+ slips in a metal-ion transporter? It does not seem to be a transient channel activity. If slippage occurred through such an 'occasional' channel that has been formed in Smf1, protons could not compete with the much higher concentration of Na^+ in the medium[66]. In addition, the pH-dependent inhibition of the divalent metal uptake by Na^+ indicates that Na^+ competes on the H^+-binding site.

We propose that the model in FIG. 3 might explain the H^+ or Na^+ slippages through Dct1 and Smf1. According to this model, the driving force and substrate pathways are normally interconnected, but can be uncoupled under certain conditions. Under conditions of neutral pH and/or neutral membrane potential, Dct1 would be in the coupled state, that is, the stoichiometry of proton:metal-ion transport would be close to 1:1 (FIG. 3a). Increasing the driving force, by either reducing the pH or increasing the negative potential, generates a proton slip, which maintains the levels of substrate transport at rates that are close to the 'normal' rates of the coupled state. However, it simultaneously increases the rate of proton movement through the transporter (FIG. 3b). A similar mechanism applies to Smf1-mediated slippage, except that here the ion that slips is Na^+. Metal-ion transport by Smf1 is also driven by PMF, and inhibition of the latter by Na^+ results in reduced metal-ion uptake.

What could be the physiological significance of the slip phenomenon in metal-ion transporters? Dct1 is the port of entry for Fe^{2+} in the duodenum, and loss-of-function mutations in the gene that encodes this protein cause anaemia[67]. Even though the interior of the duodenum is usually maintained at a neutral pH, the driving force for iron uptake by Dct1 is a proton electrochemical gradient. Excess iron and other metal ions are toxic, so a mechanism is necessary to protect cells against the excessive transportion of these elements. For example, several kinds of food product are highly enriched in iron, and eating too much of them can cause heartburn. If the excess acid that is produced reaches the duodenum together with large quantities of iron, the combination of a very high driving force and and an abundance of substrate might be deleterious, and might allow cells to take up toxic levels of metal. So, uncoupling these processes through an in-built proton slip in the mechanism of the Dct1 could protect the organism from these negative consequences.

Conclusion and perspective

The uncoupled currents that have been observed in relation to specific transporters can be interpreted by two mechanisms — occasional channel formation or a slip through the translocation pathway of the specific protein. Channels are considered to be relatively rigid entities that operate by opening and closing well-defined pores. By contrast, most transporters are flexible membrane proteins that can undergo many conformational changes, some of which are intimately linked to substrate transport across the membrane[68]. For this reason, we favour the slip mechanism — as opposed to that of occasional channel formation — to explain the deviations from strict stoichiometry between ion movements and substrate transport.

The physiological consequences of molecular slips are multifaceted. In very elaborate processes, such as photosynthesis and respiration, the partial reactions — which are often very rigid — have to be geared to each other to maximize the efficiency of the overall output of the system. However, these systems also have to be prepared to respond to abrupt changes in their driving force. To do this they incorporate a degree of flexibility in some of their operating components, which includes their ion-motive ATPase complexes; a mechanistic slip in these ATPases then fulfils this requirement.

Similarly, the abrupt changes in substrate or ion concentrations that occur in the vicinity of neurotransmitter or metal-ion transporters could be smoothed over by the existence of molecular slips. In-built slips in the mechanism of action of the respective transporters could quickly handle a dangerously high concentration of neurotransmitters in the synaptic cleft, an excess driving force in duodenal metal-ion transport, or a sudden increase in the cation concentration in the vicinity of yeast cells. These are probably only a few examples of what might be a general feature of many transporters — a feature that could be important to ensure the relative stability of cells in the context of changing environmental conditions.

Nathan Nelson and Hannah Nelson are at the Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel.
Ayelet Sacher is at The Maimen Institute for Proteasome Research, Tel Aviv University, Tel Aviv 69978, Israel.Correspondence to N.N.
e-mail: nelson@post.tau.ac.il

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