MILESTONE IN PHYSIOLOGY

JGP 100th Anniversary

Carriers, exchangers, and cotransporters in the first 100 years of the *Journal of General Physiology*

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Transporters, pumps, and channels are proteins that catalyze the movement of solutes across membranes. The single-solute carriers, coupled exchangers, and coupled cotransporters that are collectively known as transporters are distinct from conductive ion channels, water channels, and ATP-hydrolyzing pumps. The main conceptual framework for studying transporter mechanisms is the alternating access model, which comprises substrate binding and release events on each side of the permeability barrier and translocation events involving conformational changes between inward-facing and outward-facing conformational states. In 1948, the *Journal of General Physiology* began to publish work that focused on the erythrocyte glucose transporter—the first transporter to be characterized kinetically—followed by articles on the rates, stoichiometries, asymmetries, voltage dependences, and regulation of coupled exchangers and cotransporters beginning in the 1960s. After the dawn of cDNA cloning and sequencing in the 1980s, heterologous expression systems and site-directed mutagenesis allowed identification of the functional roles of specific amino acid residues. In the past two decades, structures of transport proteins have made it possible to propose specific models for transporter function at the molecular level. Here, we review the contribution of *JGP* articles to our current understanding of solute transporter mechanisms. Whether the topic has been kinetics, energetics, regulation, mutagenesis, or structure-based modeling, a common feature of these articles has been a quantitative, mechanistic approach, leading to lasting insights into the functions of transporters.

Introduction

Solute transport has been a major area of interest throughout the 100-yr history of the *Journal of General Physiology*. The purpose of this review is to summarize the contributions of *JGP* articles to our current understanding of three specific subcategories of transport: facilitated diffusion (uniport; simple carriers), coupled exchange (antiport), and coupled cotransport (symport). The related topics of epithelial transport (Palmer, 2017) and passive membrane permeation/ion selectivity (Hille, 2018) have been the subjects of excellent reviews in the Milestones in Physiology series. To minimize overlap with these articles, there is very little reference here to ion channels, diffusional permeability, or epithelial functions of transporters. There is also minimal reference to water transport, receptors, sensors, ATP-driven transport, light-driven transport, electron transport, porins, or ionophores.

Many important articles on transporters have of course appeared in other journals, but the focus here is on *JGP*, with references to other journals limited to articles representing breakthroughs in the field of transport or membrane biology in general. To limit the length of this review, it was not possible to include all *JGP* articles on transporters, especially the most widely studied systems.

First 30 yr

As described by Hille (2018), the existence of carriers with saturable binding sites for solutes was suggested in the 1930s but was not well established. Although transporters as we now define them were not yet recognized in the first 30 yr of *JGP*, many early articles described approaches or concepts that served as foundations for later studies of transporters. For example, measuring changes in intracellular or extracellular pH is central to the study of many coupled transporters. In the very first article in *JGP* (Osterhout and Haas, 1918), pH was used as an indicator of the time course of photosynthetic reactions. This article included differential equations describing the rates of photosynthesis and set an early standard for quantitative analysis of experimental data, a hallmark of *JGP*.

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In another very early article, Jacobs (1922) exposed cells from flowers, starfish eggs, and frog skin to NaCl solutions containing CO_2/HCO_3^-, NH_3/NH_4^+, or neither, and measured changes in intracellular or transcellular (for frog skin) pH using optical indicators. Even though the extracellular solution was neutral, the intracellular pH acidified when CO_2/HCO_3^- was added and alkalinized when NH_3/NH_4^+ was added. This article and others in *JGP* (Osterhout and Dorcas, 1925; Wright, 1934) established the principle that, at least in most cells, CO_2 and NH_3 are transported much more rapidly than HCO_3^- and NH_4^+.

An article by Hoagland and Davis (1923) on the cell sap of *Nitella* provided evidence for cellular pH regulation: "The hydrogen ion concentration of healthy cells was found to be approximately constant, at pH 5.2. This value was not changed even when the outside solution varied from pH 5.0 to 9.0." In the decades that have followed, the role of coupled transporters in pH homeostasis has been an important theme in *JGP*. In an another influential article on acid–base transport, Jacobs and Stewart (1942) showed that in human erythrocytes, the exchange of Cl^- for HCO_3^-, combined with the action of carbonic anhydrase, results in the effective exchange of Cl^- for OH^- via the recycling of CO_2. The Jacobs–Stewart cycle is still a relevant concept in cellular acid–base physiology.

A landmark *JGP* article by Zilversmit et al. (1943) described concepts associated with the use of tracers, including steady-state turnover rates, turnover times, and rate constants for appearance and disappearance. The article was mainly about precursor–product relations, but as the concluding sentence indicates, the ideas are relevant to transport: "These calculations take into account loss of the isotopic substance by way of breakdown or transport." The use of radioactive tracers was the first of many conceptual and technical advances beginning in the 1940s (summarized immediately below) that have led to our current understanding of transporters.

From kinetics to molecular mechanisms

From 1948 to 2018, the field of transporter research has moved from recognition of the existence of transporters all the way to structure-based models of transporter mechanisms. To provide a historical context for *JGP* articles on transporters, it is useful to summarize some of the major developments in membrane biology and transport research over the past 70 yr.

Saturation and competition: LeFevre (1948) published the first of several articles on glucose transport kinetics and the idea that glucose forms a reversible complex with a membrane-bound carrier (see below).

Active transport: By the late 1940s, tracer flux measurements had provided convincing evidence for active cation transport (Ussing, 1949). A crab nerve adenosine triphosphatase (ATPase) activity stimulated by Na^+ and K^+ was discovered by Skou (1957).

Chemiosmotic hypothesis: Mitchell (1961) proposed the chemiosmotic hypothesis for oxidative phosphorylation.

Coupled cotransport: Intestinal glucose absorption was shown to involve the coupled cotransport of Na^+ and glucose (Crane, 1962; Schultz and Zalusky, 1964). Cotransport of nutrients with H^+ was later demonstrated in bacteria (West and Mitchell, 1973).

Coupled exchange: The phenomenon of exchange diffusion was known from tracer flux measurements (Ussing, 1949). The use of ionophores later showed that the conductive Cl^- permeability of erythrocytes is much smaller than that expected from tracer fluxes (Harris and Pressman, 1967), indicating obligatory Cl^- exchange.

Recognition that transporters are proteins: Genetic studies on the expression of *Escherichia coli* lactose permease (Monod, 1966) strongly suggested that the permease is a protein; Fox and Kennedy (1965) used radiolabeling to identify and partially purify the protein.

Membrane protein cell biology and biochemistry: In the 1970s, there were several major advances in methods for studying membrane proteins: freeze fracture electron microscopy (Pinto da Silva and Branton, 1970); PAGE (Fairbanks et al., 1971); labeling and proteolysis methods for studying topology (Bretscher, 1971); measurement of lateral mobility of membrane lipids and proteins, leading to the fluid mosaic model (Singer and Nicolson, 1972); and functional reconstitution of membrane proteins in lipid vesicles (Kasahara and Hinkle, 1977) and planar lipid bilayers (Schindler and Quast, 1980).

Patch clamp method: The patch clamp method (Hamill et al., 1981) was an important advance not only for the study of ion channels but also for transporters.

cDNA sequences of transporters: With the development of recombinant DNA technology, cDNA sequences of many transporters were determined in the 1980s and 1990s.

Heterologous expression systems: Starting in the mid-1980s, heterologous expression systems and site-directed mutagenesis made it possible to examine the roles of individual amino acid residues in transporter function.

Imaging methods: Confocal microscopy and improved fluorescent labeling methods have made it possible to study the subcellular localization and trafficking of transporters.

Crystal structures: Starting with the bacterial K^+ channel (Doyle et al., 1998), the crystal structures of many pumps, channels, and transporters have been determined.

Computational methods: Advances in computational methods, especially protein structural modeling, have made it possible to study transporters at a level of detail that was not possible 20 yr ago.

Physiological and mechanistic themes

The overarching physiological theme of *JGP* articles on transporters is cellular solute homeostasis, especially with respect to nutrients, inorganic ions, and neurotransmitters. Within this overall theme, important subtopics include regulation of intracellular pH, Ca^{2+}, and cell volume. Because the focus of this article is on carriers, exchangers, and cotransporters in nonepithelial cells, some aspects of solute regulation are not discussed. For example, the section on cell volume regulation includes only transporters and not pumps, channels, or osmolyte synthesis. Similarly, the only aspect of Ca^{2+} regulation discussed here is Na^+/Ca^{2+} exchange rather than pumps, channels, receptors, and sensors. The regulation of intracellular pH is discussed in the context of transporters, with little reference to mechanisms of metabolic acid production.

A common framework for discussing transporter mechanisms is the alternating access model, in which transporters act by way

of a cycle of conformational changes between inward-facing and outward-facing states (Jardetzky, 1966). The rules governing the rates of the inward and outward conformational changes (translocation events) depend on the type of transporter. For simple carriers, the conformational change must take place either with or without substrate, thereby creating a catalytic cycle with net solute movement. For obligatory exchangers, solute influx and efflux are coupled to each other because the transporter cannot reorient rapidly in the absence of bound substrate. For cotransporters, the translocation event is rapid only if all substrates or no substrates are bound; the partially occupied transporter cannot reorient rapidly. These restrictions on the translocation event result in net cotransport of two or more substrates in the same direction with a fixed stoichiometry.

In the context of the alternating access mechanism, the theme of many *JGP* articles has been quantitative analysis of the catalytic cycle to determine transport stoichiometry, rate constants for individual events, effects of membrane potential, and intrinsic kinetic asymmetries. Precise control of ion concentrations on both sides of the membrane is a common feature of many *JGP* articles on transporters. Another theme is the study of alternative modes of transporters (e.g., exchange modes for net transporters, conductive modes for exchangers or cotransporters, and cotransport modes for exchangers), which often reveal insights into the transport mechanism. Finally, many *JGP* articles on transporters represent technical advances, e.g., improved time resolution for either measuring transport or imposing step changes in conditions.

With the above general background, the remainder of this review describes *JGP* articles over the past 70 yr on single-substrate carriers, coupled exchangers, and coupled cotransporters. The review is organized mainly according to categories of transporters, using the solute carrier (SLC) nomenclature (Hediger et al., 2004). The first category is simple carriers or uniporters, exemplified by the glucose transporter GLUT1 (SLC2). The next category is exchangers, including Na^+/Ca^{2+} (NCX, SLC8); Cl^-/CHO_3^- (SLC4); Na^+/H^+ (NHE, SLC9); and 2 Cl^-/H^+, which is a member of the Cl^- channel (CLC) family. (Although there have been many excellent *JGP* articles on Cl^- channels in the CLC family, the current review is restricted to CLCs that are exchangers.) The final category is cotransporters, including the Na^+-HCO_3^- transporter (SLC4), Na^+-K^+-2Cl^- (NKCC) and K^+-Cl^- (KCC) cotransporters (SLC12), glutamate transporter (SLC1), and γ-aminobutyric acid (GABA) transporter (SLC6). *JGP* articles on the Na^+-glucose cotransporter have been reviewed by Palmer (2017) and are not discussed further here.

Simple carriers or uniporters

A major advance toward recognizing the existence of specific solute transporters was published in *JGP* by LeFevre (1948). The article described the inhibitory effects of various substances on the influx of glycerol and glucose into human erythrocytes. The effects of Cu^{2+} and Hg^{2+} were very different for the two solutes, indicating that the two solutes are transported through distinct pathways. From a quantitative analysis of the time course of glucose influx at various concentrations, LeFevre (1948) concluded: "The behavior of the cells in the glucose solutions clearly cannot be harmonized with.... passive diffusion. If, however, it be assumed that the transfer involved temporary formation of a complex with some constituent of the cell membrane, ...there may be some limiting value, m, which the (influx) cannot exceed, regardless of the concentration gradient." The ideas of a temporary complex between substrate and transporter, and a maximum flux, independent of concentration gradient, are of course key concepts in mediated transport.

Competition between sugars for transport sites (Fig. 1 A) was demonstrated by LeFevre and Davies (1951). Addition of glucose or sorbose alone caused initial osmotic shrinkage followed by swelling as the hexose and osmotic water entered the cells. Adding glucose after sorbose caused further swelling as glucose entered the cells. However, adding sorbose after glucose did not cause further swelling (top trace), because sorbose entry was inhibited by the presence of glucose. The authors concluded: "These observations are interpreted in terms of simple equilibria between the various sugars and a hypothetical carrier molecule in the membrane, with which the sugars form a complex during their passage through the membrane." Although both the above articles used the term "active transport," LeFevre and LeFevre (1952) articulated a critical distinction between carriers and pumps: "The supposed carrier system derived from these kinetic experiments does not constitute a glucose 'pump,' and does not perform thermodynamic work in transport of glucose across the red cell surface."

In another important *JGP* article on glucose transport, Rosenberg and Wilbrandt (1957) tested the idea that if a mobile carrier mediates glucose transport, then unlabeled glucose added to one side of the membrane should induce uphill transport of ^{14}C-labeled glucose. A suspension of human erythrocytes was equilibrated with a low concentration of ^{14}C-labeled glucose, and the extracellular ^{14}C concentration was measured periodically (Fig. 1 B). At the indicated time, unlabeled glucose or mannose was added. The extracellular labeled glucose concentration would be expected to decrease slightly if there was no efflux of labeled glucose because of the dilution caused by the addition of unlabeled sugar. Instead, the extracellular labeled glucose concentration increased and then returned to the expected baseline. This countertransport phenomenon was strong evidence for a transporter that behaves as if a carrier/substrate complex effectively crosses the membrane and can bind/release substrate on either surface.

LeFevre and McGinniss (1960) extended the kinetic analysis of glucose transport by measuring the net influx and then the tracer influx in the same red cell suspension after net influx was close to equilibrium. Tracer influx was much faster than net influx (Fig. 1 C), leading to the conclusion that "net glucose movements and the tracer glucose equilibration experiments.... render untenable any interpretation of the net transfer kinetics based on a simple diffusion...." The article included a schematic of the key kinetic features of facilitated diffusion (Fig. 1 D): binding/release of substrate at both membrane surfaces and translocation of both loaded and unloaded carrier. At the time, the carrier was depicted as a small molecule that can diffuse, with or without substrate, across the membrane, but the same ideas apply to a membrane protein that undergoes conformational changes between in-

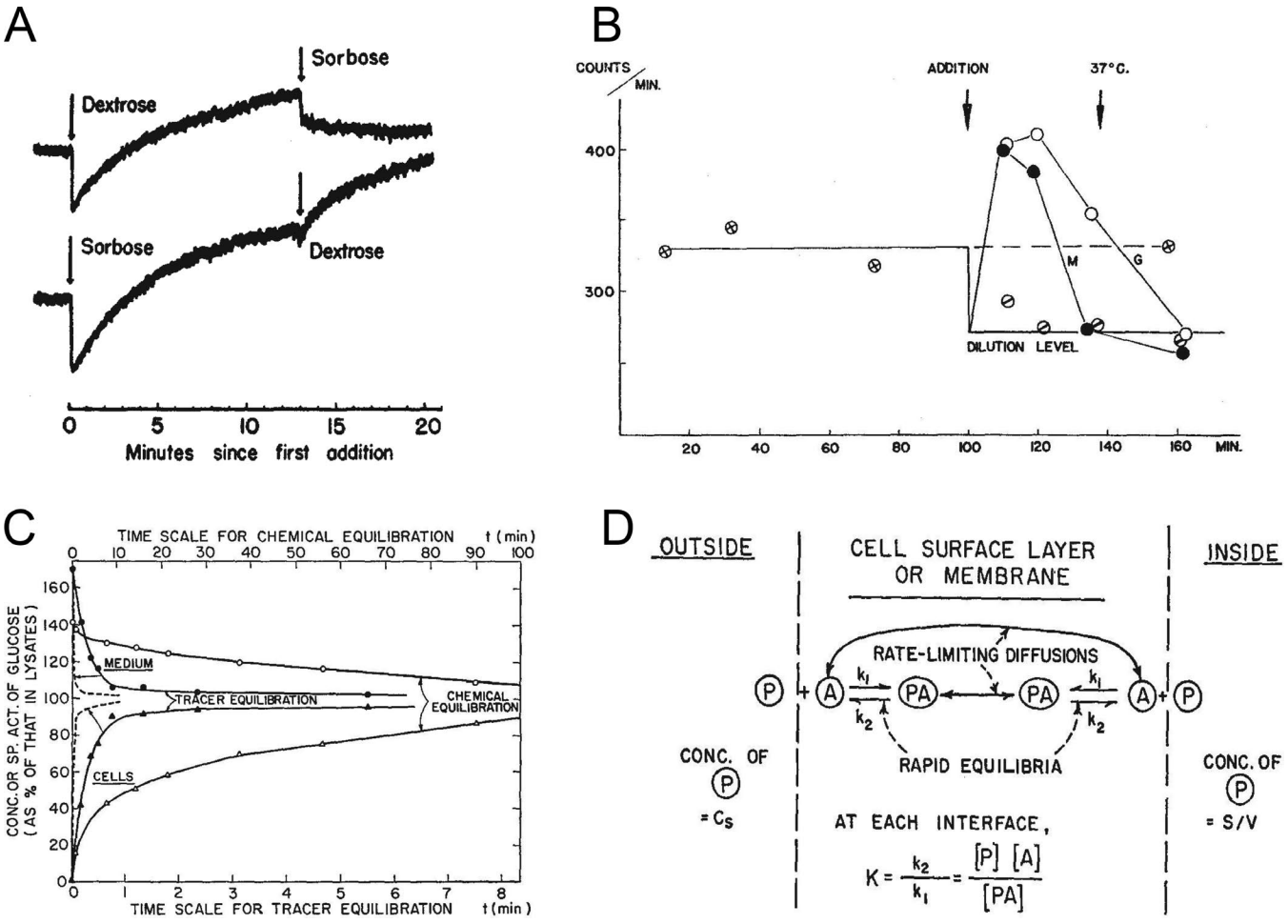

Figure 1. **Facilitated glucose transport. (A)** Competition between glucose (dextrose) and sorbose for entry into human erythrocytes (LeFevre and Davies, 1951). **(B)** Countertransport of labeled glucose caused by addition of unlabeled glucose (G) or mannose (M; Rosenberg and Wilbrandt, 1957). **(C)** Time course of tracer and net influx of glucose into human erythrocytes (LeFevre and McGinniss, 1960). Tracer influx is plotted as specific activity (SP.ACT.); net flux is plotted as glucose concentration (CONC.). **(D)** Model for the elementary events in carrier-medicated transport (LeFevre and McGinniss, 1960). P represents substrate (penetrant); A is free carrier; K is the dissociation constant for substrate binding; k_1 and k_2 are the forward and reverse rate constants for binding; C_s is extracellular substrate concentration; and S/V is intracellular substrate concentration.

ward-facing and outward-facing states (Jardetzky, 1966). The article by LeFevre and McGinniss (1960) is the oldest of over 3,600 articles generated by searching PubMed for "carrier-mediated."

As the idea of facilitated glucose transport became well established, there was increasing interest in identifying the transporter by chemical labeling. In an article on membrane labeling published the same year as the Fox and Kennedy (1965) labeling of lactose permease, Vansteveninck et al. (1965) quantified the numbers of binding sites for sulfhydryl reagents on the red blood cell membrane, inside and outside the permeability barrier. This article provided an early paradigm for quantitative, vectorial (sided) labeling of membranes, which would later be important in the biochemical characterization of membranes and in studies of other transporters.

After 1970, most work on facilitated (Na^+-independent) glucose transport was published in journals other than *JGP*, including the identification of the erythrocyte GLUT1 by reconstitution (Kasahara and Hinkle, 1977). In addition to GLUT1, other facilitated diffusion systems have been characterized in *JGP*, including those for uridine (Cabantchik and Ginsburg, 1977), urea, and ethylene glycol (Mayrand and Levitt, 1983). Very recently, Jaehme et al. (2018) demonstrated that facilitated diffusion is the mechanism of thiamine transport through PnuT, a member of the bacterial pyridine nucleotide uptake family; this mechanism is completely different from that of other bacterial thiamine transporters.

Na^+/Ca^{2+} exchanger

In the late 1960s, evidence for the coupled exchange of Na^+ for Ca^{2+} (NCX) was reported in heart muscle (Reuter and Seitz, 1968) and nerve (Baker and Blaustein, 1968). The physiological function of this exchange is to extrude Ca^{2+} across the plasma membrane by coupling Ca^{2+} efflux to Na^+ influx without the direct consumption of ATP. In the three decades that followed the initial discovery of NCX, many of its key features were established in *JGP* articles, including stoichiometry, voltage dependence, catalytic cycle, reversibility, and regulatory mechanisms.

Russell and Blaustein (1974) used the large muscle fibers of barnacle to measure the dependence of $^{45}Ca^{2+}$ efflux on extra-

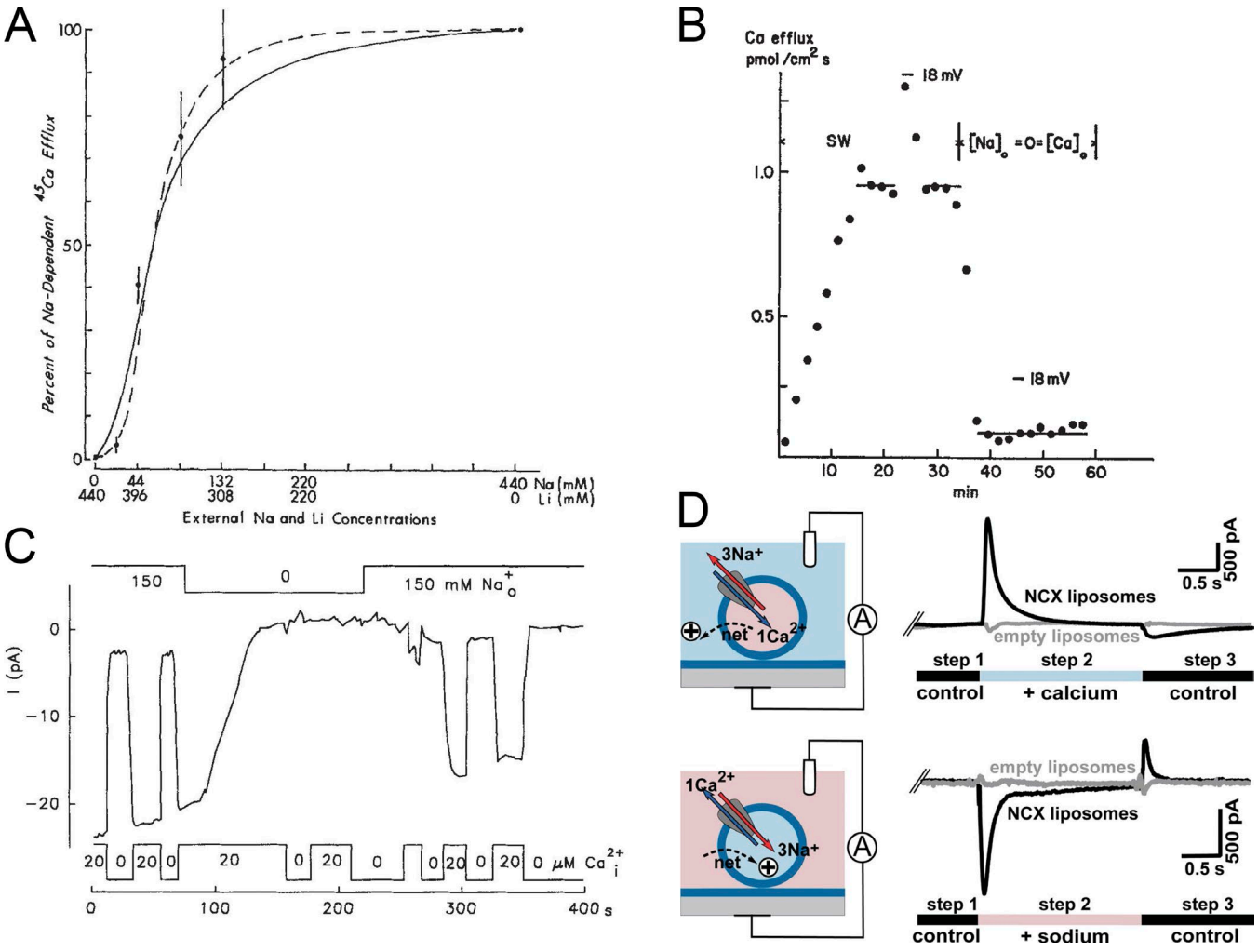

Figure 2. **Na^+/Ca^{2+} exchange. (A)** Dependence of ^{45}Ca efflux (±SEM) on extracellular Na^+ in barnacle muscle fibers (Russell and Blaustein, 1974). **(B)** Stimulation of Ca^{2+} efflux by 18 mV hyperpolarization in squid giant axon in sea water (SW) but not in a medium containing no Na^+ (Mullins and Brinley, 1975). **(C)** Na^+/Ca^{2+} exchange currents (I; pA) in guinea pig heart muscle measured in perfused giant patch (Hilgemann et al., 1992a). **(D)** Na^+/Ca^{2+} exchange currents in NCX from *Methanococcus jannaschii*, as measured using reconstituted vesicles and solid-supported membrane technology (Barthmes et al., 2016).

cellular Na^+ (Fig. 2 A). They found that "the relation between the Na_o-dependent Ca efflux and external Na concentration is sigmoid, and suggests that two, or more likely three, external Na^+ ions may activate the efflux of one Ca^{2+}. With a three-for-one Na–Ca exchange, the Na electrochemical gradient may be able to supply sufficient energy to maintain the Ca gradient in these fibers."

Dipolo (1973) and DiPolo (1974) showed that NCX in squid giant axons is modulated by the ATP concentration. Also in squid axon, Mullins and Brinley (1975) varied the intracellular-free Ca^{2+} concentration by dialysis with EGTA and clamped the membrane potential during $^{45}Ca^{2+}$ efflux measurements (Fig. 2 B). They showed that external Na^+-dependent $^{45}Ca^{2+}$ efflux is stimulated by hyperpolarization, indicating that $^{45}Ca^{2+}$ efflux is associated with net influx of positive charge, consistent with 3:1 Na^+/Ca^{2+} exchange. As part of the study of NCX, new methods were developed to quantify and control cellular Ca^{2+} buffering and free cytosolic Ca^{2+} (Dipolo et al., 1976; Brinley et al., 1977, 1978); the principles of Ca^{2+} buffering established in squid axon have had broad application in other systems.

DiPolo (1979) devised a method for measuring $^{45}Ca^{2+}$ influx to study the "reverse" mode of NCX in internally dialyzed squid axons. By varying the intracellular Na^+ and Ca^{2+} concentrations, he demonstrated that in the nominal absence of internal Ca^{2+}, there is no Ca^{2+} influx stimulated by internal Na^+, indicating that cytosolic Ca^{2+} is a regulator of the exchanger in addition to being a substrate. Several years later, DiPolo and Beaugé (1987) studied the reverse mode of exchange using $^{22}Na^+$ efflux and again found that "the level of ionized Ca_i modulates the velocity of the Na/Ca exchange working in the reverse mode."

The studies of NCX in squid axon and barnacle muscle were followed by a series of equally rigorous *JGP* articles on NCX in heart muscle. Horackova and Vassort (1979) showed that tonic tension elicited by depolarization of frog atrial muscle is regulated by an exchange process with Na^+:Ca^{2+} stoichiometry of at least 3:1. Miura and Kimura (1989) used whole-cell patch clamping and intracellular perfusion of guinea pig single ventricular cells to quantify the kinetics of the NCX current. From the half-maximal concentrations of internal Na^+ and Ca^{2+}, they determined that substrate binding affinities are "at least apparently

asymmetrical between the inside and outside of the membrane" and that "the exchanger is fully activated in the physiological calcium concentration range."

Early kinetic models of NCX were generally the equivalent of a mobile carrier/alternating access model in which the unoccupied carrier does not translocate rapidly (Russell and Blaustein, 1974; Horackova and Vassort, 1979). Other models were proposed (Mullins, 1977), but by the 1980s, alternating access was widely accepted as the working model of NCX.

In 1992, Hilgemann and coworkers published three *JGP* articles that produced new insights regarding the individual steps in the catalytic cycle and regulation of NCX. They used guinea pig cardiac myocytes and the perfused giant patch clamp method, which allows very rapid replacement of the cytoplasmic medium and slower (but complete) replacement of the extracellular medium (Fig. 2 C). Hilgemann et al. (1992a) measured the time course of inactivation of the reverse exchange mode after a step increase in cytoplasmic [Na^+]. From a detailed analysis of the kinetics of inactivation, they developed a model in which the exchanger enters the inactivated state from an inward-facing state that has three bound Na^+ ions. Hilgemann et al. (1992b) then showed that both MgATP and cytoplasmic Ca^{2+} slow the time course of Na^+-dependent inactivation and accelerate the recovery from inactivation. Finally, Matsuoka and Hilgemann (1992) did a thorough characterization of the kinetics of the transport cycle after removing the inactivation machinery with chymotrypsin. The results were all consistent with an alternating access-type model involving occlusion/deocclusion of both Na^+ and Ca^{2+}, with the former being more voltage-dependent.

Matsuoka et al. (1995) used excised membrane patches from *Xenopus* oocytes expressing NCX to further investigate the regulation of the exchanger. By mutating aspartate residues in a region suspected of containing high-affinity Ca^{2+} binding sites, they identified residues important for regulatory Ca^{2+} binding. The low-affinity mutants made it possible to study Ca^{2+} regulation of the exchanger operating in the forward (Ca^{2+} efflux) mode; previously, such experiments were compromised by the simultaneous presence of high-affinity substrate and regulatory Ca^{2+} sites on the same side of the membrane. This work demonstrated that, as is true of the reverse mode, "forward mode Na^+–Ca^{2+} exchange is modulated by Ca^{2+} at the regulatory site. In vivo, with Ca^{2+} at the submicromolar level, secondary Ca^{2+} regulation may exert an important influence on Ca^{2+} extrusion." Matsuoka et al. (1997) used the same system to characterize the exchanger inhibitory peptide (XIP) region. The experiments demonstrated that XIP is involved not only in Na^+-dependent inactivation but also in Ca^{2+} regulation of the exchanger.

Weber et al. (2001) advanced the study of regulation of NCX in intact ferret cardiac myocytes by using the exchanger itself, as driven by the clamped membrane voltage, to control the entry and exit of Ca^{2+} and thereby control intracellular [Ca^{2+}]. These studies showed "for the first time allosteric regulation in intact myocytes at physiological $[Ca^{2+}]_i$, and, under conditions where the NCX is able to dynamically control $[Ca^{2+}]_i$."

NCX_Mj from *Methanococcus jannaschii* is the only Na^+/Ca^{2+} exchanger for which a crystal structure has been determined, and it is important to know the functional properties of the protein. Barthmes et al. (2016) recently characterized the electrical properties of NCX_Mj, using solid-supported membrane technology to measure currents associated with Na^+/Ca^{2+} exchange in both directions (Fig. 2 D) and estimate apparent substrate affinities. Very recently, Shlosman et al. (2018), using tracer flux measurements with NCX_Mj reconstituted into proteoliposomes, found that the exchanger has a 3:1 Na^+:Ca^{2+} stoichiometry. The fact that the basic catalytic function is similar to that of mammalian NCX indicates that NCX_Mj promises to be an important system for the study of the molecular details of the exchange process.

Red blood cell Cl^-/HCO_3^- exchanger

Until the late 1960s, red cell Cl^- transport was believed to be conductive, but the low rate of net KCl efflux from gramicidin-treated red cells (Harris and Pressman, 1967) implied that almost all of the $^{36}Cl^-$ flux in red cells represents obligatory $^{36}Cl^-/Cl^-$ exchange. The exchange is mediated by the transporter known as band 3 or anion exchanger 1 (AE1;SLC4A1), the physiological function of which is the exchange of Cl^- for HCO_3^- as one of the steps in CO_2 transport. In tissue capillaries, CO_2 diffuses into red cells and is converted by carbonic anhydrase into HCO_3^-, which exchanges with extracellular Cl^- to increase the CO_2 carrying capacity of blood.

Definitive evidence for carrier-mediated Cl^- transport in red cells was published in *JGP* by Gunn et al. (1973), who measured $^{36}Cl^-/Cl^-$ exchange at 0° with a rapid sampling method. They used combinations of Na-acetate, NH_4-acetate, and NH_4Cl to vary the intracellular and extracellular Cl^- concentration and demonstrated for the first time that red cell Cl^-/Cl^- exchange is a saturable process (Fig. 3 A). They concluded that "a specific interaction of these anions (Cl^- and HCO_3^-) with a component of the erythrocyte membrane is involved in their transport." Cabantchik and Rothstein (1974) shortly afterward showed that the anion transport inhibitor H_2DIDS labels the band 3 protein. The experimental approach for labeling band 3 had been described earlier in a *JGP* article by Knauf and Rothstein (1971).

The demonstration that red cell Cl^- exchange is carrier mediated and the identification of the transport protein stimulated a period of intense work on this system. Dalmark (1976) used nystatin to vary red cell ion contents to estimate relative affinities of the halides and HCO_3^-. Brazy and Gunn (1976) showed that the loop diuretic furosemide inhibits red cell Cl^-/Cl^- exchange. In a very careful study using a combination of manual sampling and a flow tube, Brahm (1977) measured red cell Cl^-/Cl^- exchange over a wide temperature range and found that the activation energy changes when the turnover number reaches a critical rate.

Gunn and Fröhlich (1979) did a detailed study of the symmetry of red cell anion exchange, using Cl^- and Br^- as exchange partners. From the effects of external Cl^- on $^{82}Br^-$ efflux and effects of external Br^- on $^{36}Cl^-$ efflux, they showed that the transporter has asymmetric apparent substrate affinities (external Cl^- $K_{1/2}$ lower than internal) and kinetics consistent with a ping-pong mechanism, which is a version of the alternating access model in which the binding, translocation, and release of one exchange partner is followed by the binding, translocation, and release of the other exchange partner in the opposite direction.

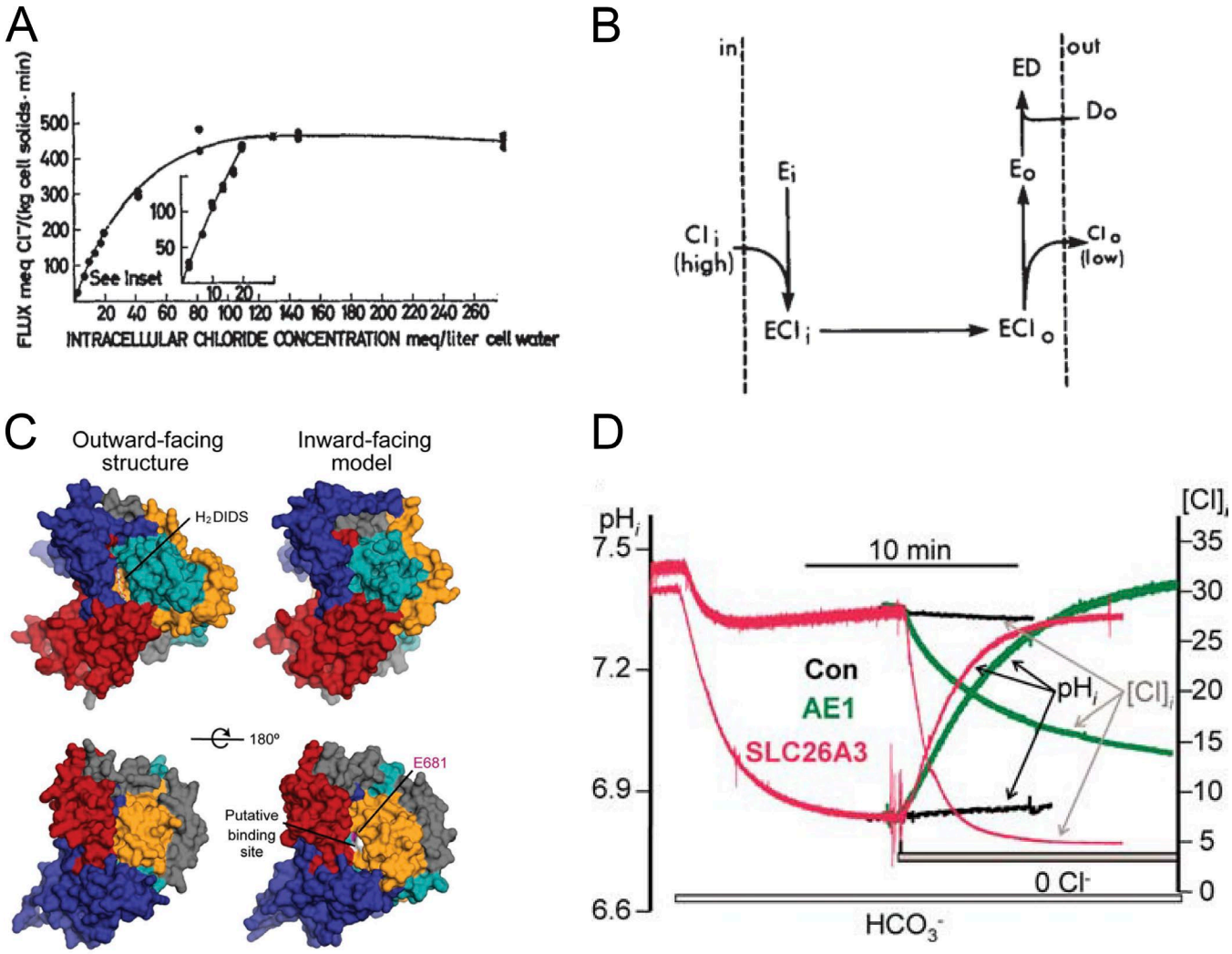

Figure 3. **Na^+-independent Cl^-/HCO_3^- exchange. (A)** First demonstration of saturation of $^{36}Cl^-$–Cl^- exchange in human erythrocytes (Gunn et al., 1973). **(B)** Schematic representation of the basic features of alternating access 1:1 exchange with inhibitor H_2DIDS (Do) binding to the outward-facing state resulting in the complex ED (Furuya et al., 1984). E is the unoccupied transporter; ECl is the transporter occupied by Cl^- at the substrate binding site; and subscripts i and o respectively denote intracellular and extracellular. **(C)** Outward-facing state of human AE1 membrane domain from published crystal structure and inward-facing state based on repeat-swap modeling (Ficici et al., 2017). **(D)** Cl^-/HCO_3^- exchange mediated by AE1 (green; 1 Cl^-:1 HCO_3^-) and SLC26A3 (red; 2 Cl^-:1 HCO_3^-) in *Xenopus* oocytes, as determined by changes in intracellular pH and $[Cl^-]$ (Shcheynikov et al., 2006). Black traces are from control oocytes (Con) expressing neither protein.

A direct test of the alternating access mechanism for Cl^- exchange is possible in red cells because of the abundance of AE1. Jennings (1982) loaded human red cell ghosts with ~3 times as much $^{36}Cl^-$ as the number of copies of AE1 (~10^6 protomers per cell). Efflux of $^{36}Cl^-$ into a medium containing no rapidly penetrating anion consisted of a burst of ~800,000 Cl^- ions, or one Cl^- ion per initially inward-facing AE1 protomer, followed by much slower further efflux. This was the first measurement of a partial catalytic cycle in a coupled exchanger and demonstrated that internal Cl^- binding, efflux, and release can take place without anion influx, as predicted by the alternating access mechanism.

Further evidence for alternating access and transport asymmetry came from the inhibitor studies of Knauf and coworkers (Furuya et al., 1984), who showed that the inhibitory potency of H_2DIDS is enhanced by an outward Cl^- gradient, as expected if the inhibitor binds preferentially to the outward-facing transporter and the gradient drives transporters into the outward-facing state by mass action (Fig. 3 B). Quantitative analysis of the effects of Cl^- gradients on the inhibitory potency of niflumic acid indicated that with symmetric $[Cl^-]$, the majority of both loaded and unloaded transporters face inward (Knauf and Mann, 1984). The kinetic scheme in Fig. 3 B reiterates the requirement that in a coupled exchanger with an alternating access mechanism, the unoccupied transporter cannot undergo the conformational change between inward-facing and outward-facing states.

As is true of GLUT1 and NCX, the study of alternate modes of AE1 has been instructive. One such mode of red cell AE1 is conductive anion transport. Knauf et al. (1977) demonstrated that graded irreversible inhibition with DIDS causes parallel decreases in anion exchange and Cl^- conductance, indicating that although >99% of the $^{36}Cl^-$ flux is electroneutral, band 3 also mediates conductive Cl^- flux. A possible mechanism of outward

conductive Cl^- transport is "slippage," or return of the unloaded transporter to the inward-facing state in the absence of bound Cl^-. Knauf et al. (1983) and Fröhlich (1984) examined and ruled out slippage as a significant mechanism of AE1-mediated conductance on the basis of the effects of Cl^- concentration on the conductance. This work placed a low upper limit on the rate at which the unloaded transporter can reorient inward.

Another mode of AE1 is the cotransport of H^+ and SO_4^{2-}. Milanick and Gunn (1982) measured the effects of extracellular SO_4^{2-} and H^+ on $^{36}Cl^-/Cl^-$ exchange to determine the order of the binding of SO_4^{2-} and H^+. Kinetic analysis indicated that H^+ and SO_4^{2-} bind in either order to the outward-facing transporter and that binding of one ion increases the affinity of the other by 10-fold. Subsequent work on the H^+-$SO_4^=$ cotransport site using either chemical modification (Jennings and Al-Rhaiyel, 1988) or mutagenesis (Chernova et al., 1997) of a specific glutamate residue (human E681) suggested that the negative charge on E681 crosses the permeability barrier in the anion translocation event.

In the past 20 yr, progress toward a molecular-level understanding of the AE1 exchange process was limited by lack of structural information until Arakawa et al. (2015) published a crystal structure of human AE1 membrane domain in a conformation approximating the outward-facing state. In a recent *JGP* article, Ficici et al. (2017) used repeat-swap homology modeling to produce a predicted structure of the inward-facing state (Fig. 3 C). The model indicates that AE1 may transport anions by way of an elevator-like mechanism.

Anion exchange protein 2

AE1 is not a system for regulating intracellular pH in erythrocytes; monovalent anion exchange through erythrocyte AE1 has very little pH dependence in the neutral range (Gunn et al., 1973; Wieth and Bjerrum, 1982). In contrast, the related anion exchanger AE2, which is widely expressed, is activated by alkaline intracellular pH, as expected if its physiological function is to extrude HCO_3^- and regulate cytosolic pH. Stewart et al. (2002) used *Xenopus* oocyte expression to investigate the molecular origin of the effects of pH on AE2-mediated anion transport. They localized a pH regulatory region in the N-terminal cytoplasmic domain and found that "a limited, highly conserved region of the AE2 NH_2-terminal cytoplasmic domain is involved in AE2 regulation by both pH_i and pH_o." Other HCO_3^- transporters involved in cellular pH regulation are described below.

Other Na^+-independent anion exchangers

Another family of anion transporters is the SLC26 transporters, which include proteins associated with several human genetic diseases. An important advance in understanding the mechanism of SLC26 transporters was the work of Shcheynikov et al. (2006) on the stoichiometries of slc26a3 (responsible for congenital Cl^- diarrhea) and slc26a6 (apical anion exchanger in several epithelia). Using expression in *Xenopus* oocytes and HEK293 cells, they showed that slc26a3 mediates an electrogenic 2 Cl^-/1 HCO_3^- exchange (Fig. 3 D), and slc26a6 mediates an electrogenic 1 Cl^-/2 HCO_3^- exchange. Prior to this work, the stoichiometry of Cl^- HCO_3^- exchange had not been known for any member of the SLC26 family.

SLC26 transporters, in addition to being coupled exchangers, exhibit channel-like modes. Ohana et al. (2011) modeled Slc26a6 and found potential structural similarity with CLC transporters. They identified a glutamate residue in Slc26a6 that is predicted to have the same spatial orientation as a critical carboxyl residue, E148, in the bacterial exchanger CLC-ec1, which is discussed further below.

Regulation of cytoplasmic pH by acid extrusion

Early *JGP* articles established that in most cells, CO_2 permeability is higher than that of HCO_3^- (see First 30 yr). Near the end of the sixth decade of *JGP*, a landmark article by Boron and De Weer (1976) described the response of intracellular pH of squid giant axon to sustained exposures to extracellular CO_2/HCO_3^-. To analyze the time course of intracellular pH, they developed a mathematical model that included the permeability not only of CO_2 but also of HCO_3^-. Experimentally, the intracellular pH of squid axons acidified as expected following addition of CO_2/HCO_3^- (Fig. 4 A), but the pH slowly began to recover in the continued presence of CO_2/HCO_3^-, indicating the presence of a pH-regulatory acid extrusion process. The ideas described in this article about responses of cells to acid or alkaline loads have since been applied widely in many different biological systems.

Na^+-dependent Cl^--HCO_3^- exchanger

In the years after the discovery by Boron and De Weer (1976) of an acid-extruding process in squid axon, several *JGP* articles characterized the ion transport processes responsible for acid extrusion. Boron and Russell (1983), using a perfusion chamber with pH electrodes, voltage electrode, and internal dialysis (Fig. 4 B), showed that the acid extrusion system in squid axon "mediates the obligate net influx of HCO_3^- (or equivalent species) and Na^+ and the net efflux of Cl^- in the stoichiometry of 2:1:1." This Na^+-dependent Cl^-/HCO_3^- exchange (NDCBE) could take place by various combinations of Na^+, H^+, Cl^-, HCO_3^-, and $CO_3^=$ fluxes (Fig. 4 C). A functionally similar system was shown to mediate acid extrusion in barnacle muscle (Russell et al., 1983) and embryonic chick heart cells (Liu et al., 1990). Boron (1985) measured the extracellular Na^+ and HCO_3^- dependences of the acid extrusion rate in squid axon; the data were consistent with the idea that the actual extracellular substrate is the monovalent anion $NaCO_3^-$ resulting from the ion pairing of Na^+ and $CO_3^=$. A recent finding in *JGP* is that the SLC4 family member Ae4 carries out cation (Na^+ or K^+)-dependent Cl^-/HCO_3^- exchange; the physiological function of the exchange may be to drive Cl^- influx in secretory cells (Peña-Münzenmayer et al., 2016).

Na^+-HCO_3^- cotransporter

The SLC4 family includes Cl^-/HCO_3^- exchangers as well as several Na^+-HCO_3^- cotransporters (NBC). The first evidence for Na^+-HCO_3^- cotransport, the function of which in the kidney is proximal tubular HCO_3^- reabsorption, was published in *JGP* (Boron and Boulpaep, 1983), but there have been only a few *JGP* articles on NBC in nonepithelial cells. Deitmer (1991) demonstrated that glial cells in segmental ganglia of the leech exhibit stilbene disulfonate–sensitive, Cl^--independent, electrogenic Na^+-HCO_3^- cotransport. Optical measurements of intracellular

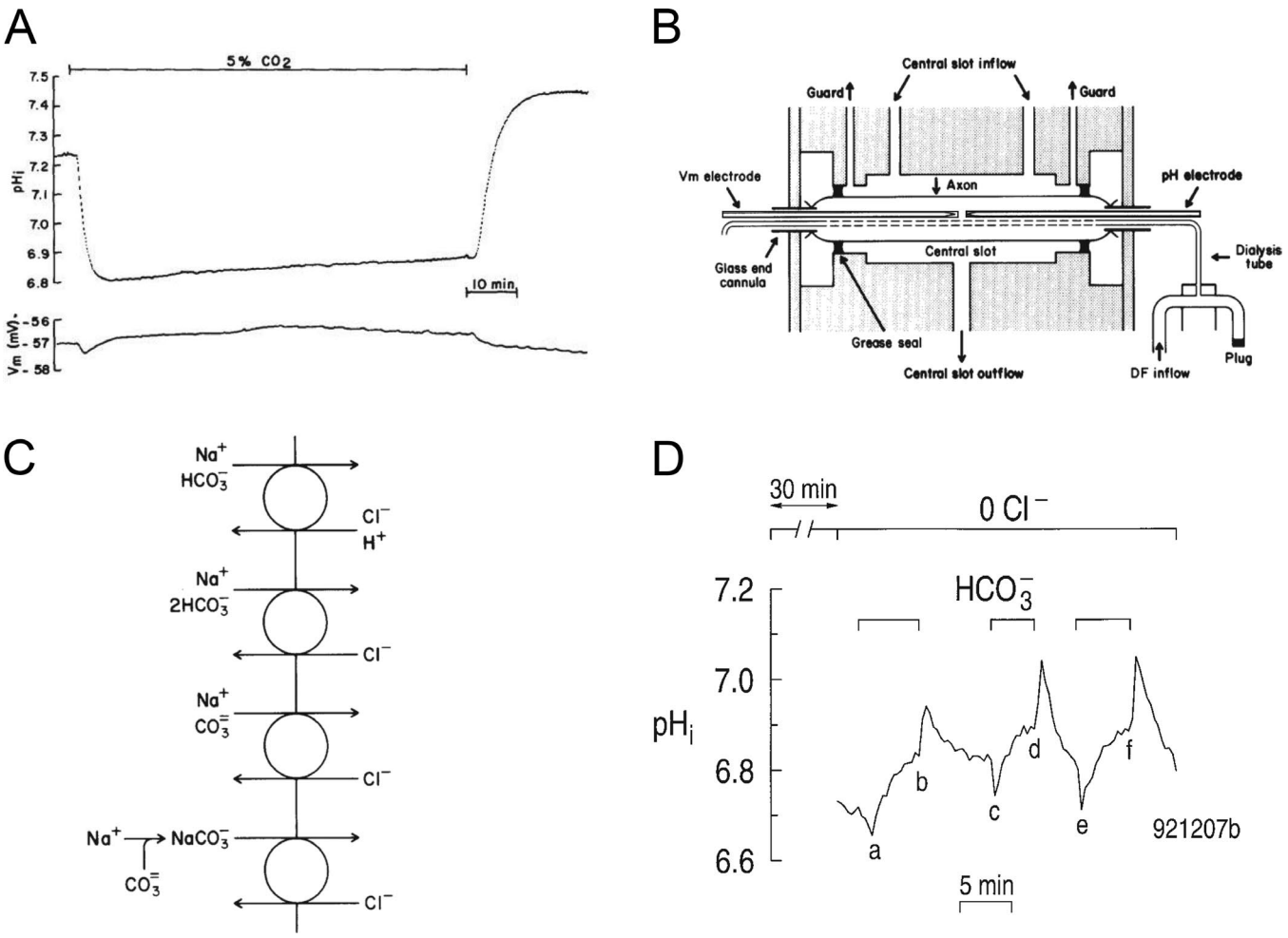

Figure 4. **Intracellular pH regulation and Na^+-dependent HCO_3^- transport. (A)** Recovery of intracellular pH in squid giant axons during prolonged exposure to 5% CO_2 with simultaneous measurement of membrane potential (V_m; Boron and De Weer, 1976). **(B)** Chamber for perfusion of dialysis fluid (DF) and the simultaneous measurement of tracer flux, membrane potential, and intracellular pH in squid giant axon (Boron and Russell, 1983). **(C)** Possible modes of transport for NDCBE (Boron and Russell, 1983). **(D)** Demonstration of Cl^--independent Na^+-HCO_3^- cotransport in rat hippocampal astrocytes (Bevensee et al., 1997b).

pH demonstrated that in addition to an amiloride-sensitive Na^+/H^+ exchanger (discussed further below), a significant acid extruder in rat hippocampal astrocytes is a Cl^--independent electrogenic 1:2 Na^+-HCO_3^- cotransporter (Bevensee et al., 1997a,b; Fig. 4 D). After the amino acid sequences of NBCs had become known, McAlear et al. (2006) compared the functional properties of three variants of rat electrogenic NBCe1 having different N-terminal cytoplasmic domains. Naturally occurring variants and versions with truncated cytoplasmic domains were expressed in *Xenopus* oocytes. The HCO_3^--induced currents indicate that the cytoplasmic domains have significant and diverse modulatory effects on the current.

Na^+/H^+ exchanger

The obligatory exchange of Na^+ for H^+ (NHE) is a major mechanism for cellular acid extrusion and pH homeostasis. Many important *JGP* articles on both epithelial (Palmer, 2017) and nonepithelial NHE were published in the 1980s. Grinstein et al. (1984a) showed that acidification of rat thymic lymphocyte cytosol stimulates H^+ efflux (Fig. 5 A) that requires extracellular Na^+ and is inhibited by amiloride. Grinstein et al. (1984b) followed these results with $^{22}Na^+$ flux measurements (Fig. 5 B) demonstrating that NHE can take place in either direction, has a 1:1 stoichiometry, and is activated by cytoplasmic but not extracellular acidification. Piwnica-Worms et al. (1985) showed that cultured chick heart cells exhibit amiloride-sensitive NHE that has a 1:1 stoichiometry and is activated by intracellular acidification. Simchowitz and Roos (1985) demonstrated Na^+-dependent, amiloride-sensitive pH recovery from an acid load in human neutrophils. Green et al. (1988) determined the Na^+ and H^+ dependences of NHE in osteoblasts. Other *JGP* articles on NHE concern regulation of exchange by mechanisms other than intracellular pH, including O_2 (Motais et al., 1987), catecholamines (Borgese et al., 1986), and cell volume (see below).

In a very recent *JGP* article, Launikonis et al. (2018) describe a novel combination of mechanical skinning and confocal microscopy with fast time resolution to characterize NHE in rat skeletal muscle t-tubules. Fig. 5 C shows a confocal image of a fluorescent pH indicator dye trapped in the t-tubules of a skinned fiber. The lumen of the sealed t-tubule had been the original extracellular medium. Rapid changes in the medium surrounding the sealed t-tubule (cytoplasmic side of the mem-

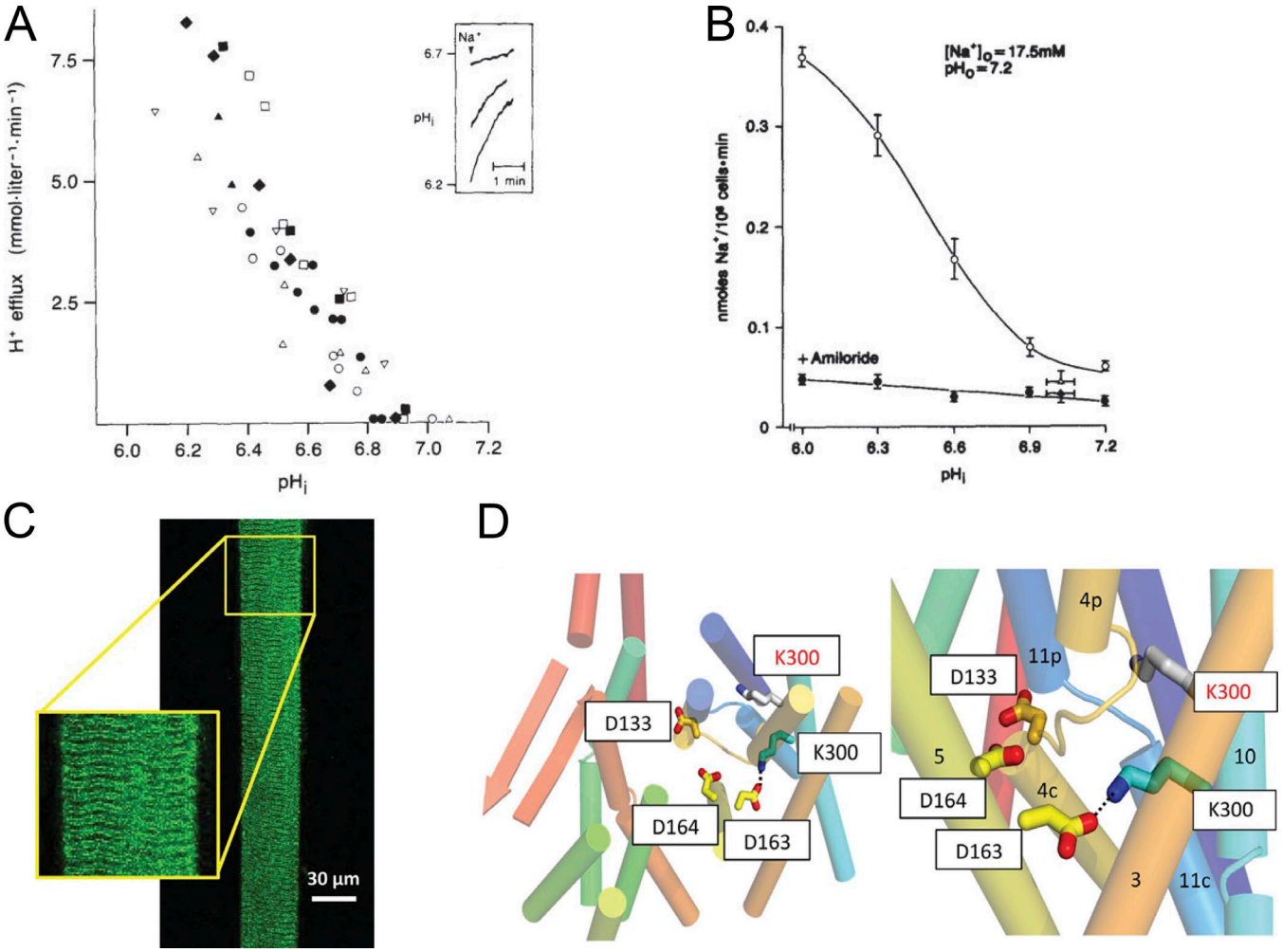

Figure 5. **Na⁺/H⁺ exchange. (A)** Intracellular pH dependence of H^+ efflux mediated Na^+/H^+ exchange in rat thymic lymphocytes (Grinstein et al., 1984a). **(B)** Intracellular pH dependence of ouabain-insensitive Na^+ efflux (mean ± SEM; Grinstein et al., 1984b). **(C)** Confocal micrograph of mechanically skinned, dye-loaded muscle t-tubules used in measurements of Na^+/H^+ exchange (Launikonis et al., 2018). **(D)** Schematic of the structure of *E. coli* Na^+/H^+ exchanger NhaA, showing the salt bridge between D163 and K300 near the center of the transport pathway (Lee et al., 2014).

brane) and confocal optical recording of fluorescence made it possible, for the first time, to detect and quantitatively characterize NHE in muscle t-tubules.

Recent *JGP* articles on NHE include structure-based studies. Lee et al. (2014) reported a new crystal structure of the *E. coli* Na^+/H^+ exchanger NhaA. The new structure revealed a salt bridge between conserved residues D163 and K300 (Fig. 5 D) that was not observed in the only other crystal structure of NhaA. Molecular dynamics simulations suggested "that the transport mechanism involves Asp163 switching between forming a salt bridge with Lys300 and interacting with the sodium ion." This work moves the study of Na^+/H^+ exchange closer to a molecular-level understanding.

$2Cl^-/H^+$ exchangers in the CLC family

Soon after the crystal structure of a bacterial CLC was determined (Dutzler et al., 2002), Accardi and Miller (2004) showed that *E. coli* CLC (CLCec1) is actually not a Cl^- channel but instead mediates the coupled exchange of 2 Cl^- for H^+. The function of CLCec1 is to expel H^+ during the extreme acid resistance response that allows enteric bacteria to survive exposure to gastric acid (Accardi and Miller, 2004).

As with other exchangers, a major question for CLCec1 is how the movement of Cl^- in one direction is coupled to the movement of H^+ in the opposite direction. In several *JGP* articles, Miller, Accardi, and coworkers used a combination of mutagenesis, reconstitution, current recordings, and $^{36}Cl^-$ flux measurements to advance our understanding of the coupling between Cl^- and H^+ fluxes. The conceptual framework is again the alternating access model. Purified CLCe1, reconstituted at high density in proteoliposomes and fused into lipid bilayers, mediates a current driven by the Cl^- gradient at zero voltage (Fig. 6 A); current increments represent individual fusion events (Accardi et al., 2004). Removal of the negative charge on E148, which normally blocks the access pathway for extracellular Cl^-, eliminates the coupling between H^+ and Cl^- movements (Accardi et al., 2004). Mutation of inward-facing aspartate and glutamate residues identifies E203 as a critical site; mutating this residue to glutamine eliminates coupling of H^+ and Cl^- fluxes (Accardi et al., 2005). The locations of E148 and E203 relative to the Cl^- binding sites suggest

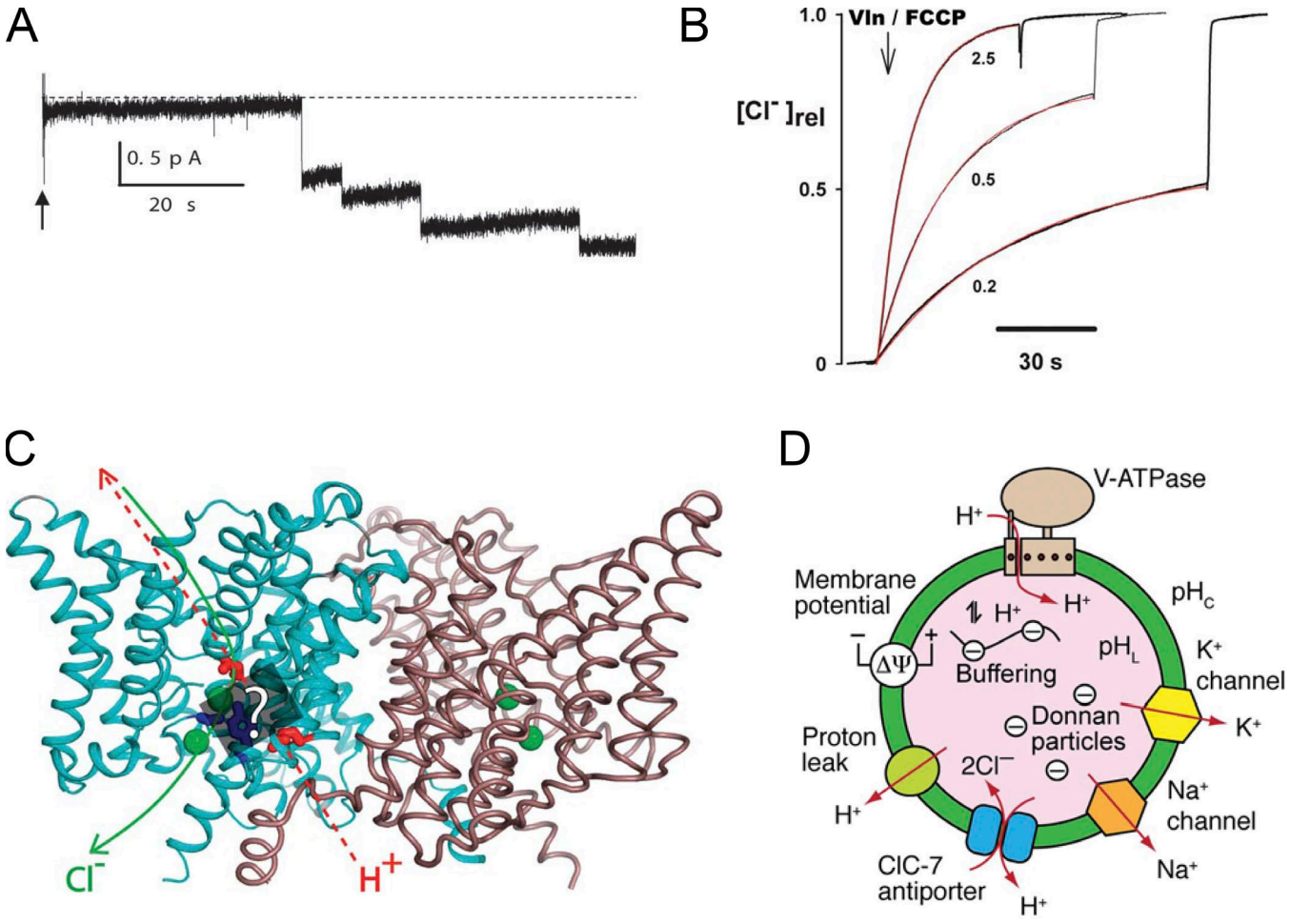

Figure 6. **CLC $2Cl^-/H^+$ exchange. (A)** Recording showing currents at zero voltage driven by the Cl^- gradient during successive fusions of CLCec1-containing proteoliposomes with a planar lipid bilayer (Accardi et al., 2004). **(B)** Time course of Cl^- efflux from CLCec1-containing proteoliposomes with three different protein concentrations following addition of valinomycin and FCCP to produce maximum efflux conditions (Walden et al., 2007). **(C)** CLCec1 dimer showing a bifurcated pathway for Cl^- and H^+ (Accardi et al., 2005). **(D)** Transporters, pumps, and channels in a model of lysosome acidification (Ishida et al., 2013).

a bifurcated transport pathway in which both Cl^- and H^+ have similar pathways to the extracellular medium but very different pathways to the intracellular medium (Fig. 6 C). Walden et al. (2007) used the same experimental system to show that mutating residues near a conserved tyrosine residue near the center of the transport pathway uncouples Cl^-/H^+ exchange. The uncoupling does not affect the unitary Cl^- transport rate, which was estimated much more precisely than previously (Fig. 6 B).

In mammals, many CLCs are Cl^- channels, but some are $2Cl^-/H^+$ exchangers; the function of these exchangers is to neutralize the positive charge pumped into acidic organelles by the H^+-ATPase. An important question regarding these exchangers is whether a $2Cl^-/H^+$ exchanger, a Cl^- channel, or some other channel is a better pathway for neutralizing the charge pumped into acid organelles by the H^+-ATPase. Ishida et al. (2013) experimentally determined the current voltage relation for mammalian lysosomal CLC7, which is an exchanger. They then used this information and computer simulations to determine the steady-state luminal pH and membrane potential for a model lysosome with a fixed passive proton permeability, 300 copies of V-ATPase, and various possible counterion pathways, including CLC-7 exchanger or channels for Cl^-, K^+, or Na^+ (Fig. 6 D). The model is an extension of the earlier model in a *JGP* article by Grabe and Oster (2001). The simulations indicated that the $2Cl^-/H^+$ exchanger results in a final luminal pH that is at least 0.5 units lower than that found if a Cl^- channel is the counterion pathway. The simulations also predict that CLC7 is a more effective counterion pathway than either Na^+ or K^+ channels. This is an important insight regarding the physiological function of mammalian $2Cl^-/H^+$ exchangers.

Coupled transporters in cell volume regulation

In addition to being a leading journal for studies of transporters involved in intracellular pH regulation, there have been many important *JGP* articles on the regulation of cell volume. An early landmark article by Tosteson and Hoffman (1960) established the pump-leak paradigm for regulating cellular cation composition and cell volume. The steady-state ion and water contents in both high-K^+ and low-K^+ sheep red cells were shown to be the result of a balance between uphill transport by way of the Na^+, K^+ pump and downhill transport by way of what were then assumed to be diffusional leaks with fixed permeabilities.

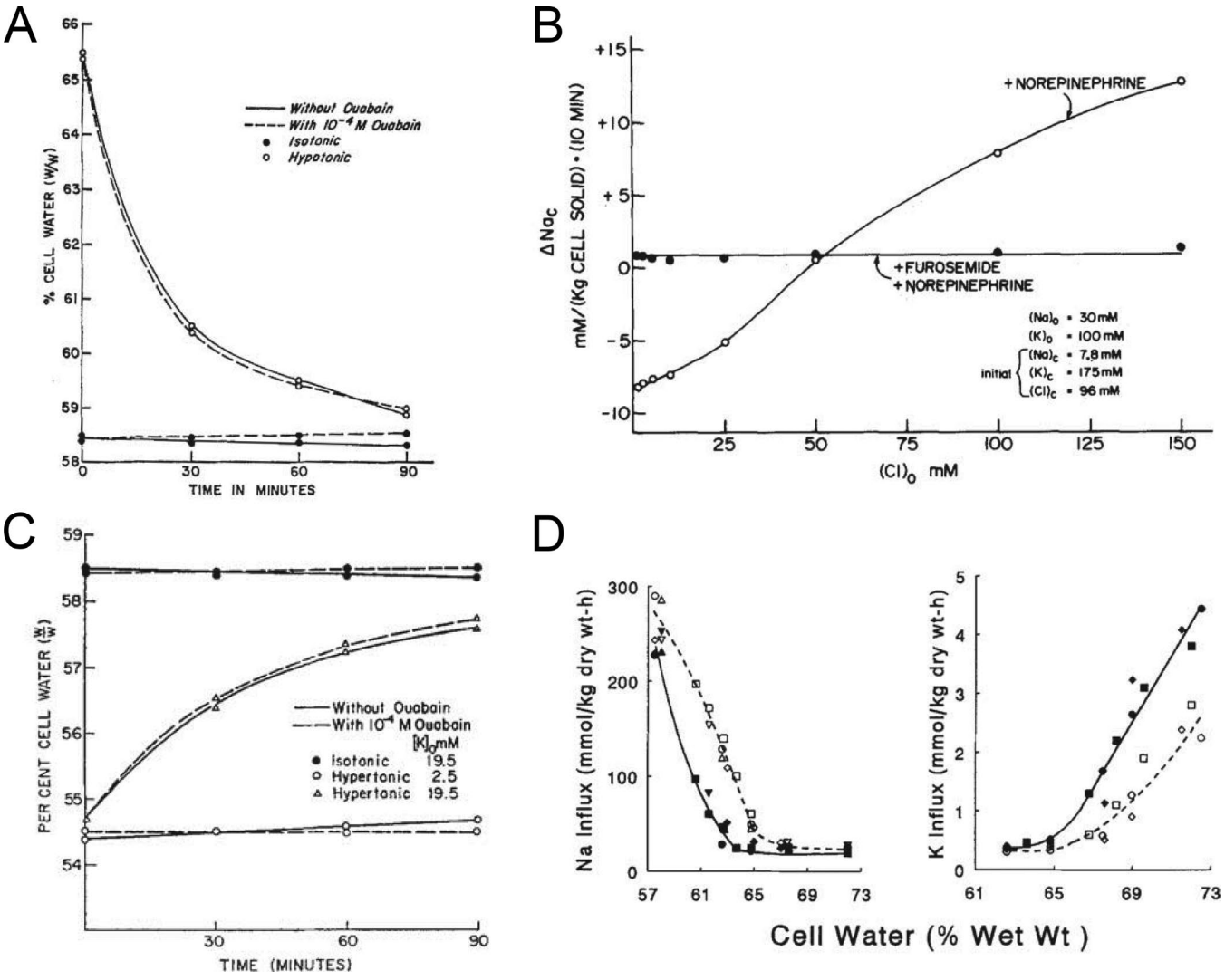

Figure 7. **Red blood cell volume regulation, NHE, NKCC, and KCC. (A)** Time course of RVD in duck red cells (Kregenow, 1971a). **(B)** Change in intracellular Na^+ driven by the Cl^- gradient in in duck red cells treated with norepinephrine to stimulate NKCC (Haas et al., 1982). **(C)** Time course of RVI in duck red cells (Kregenow, 1971b). **(D)** Effect of okadaic acid (open symbols) on the cell volume dependence of NHE and KCC in dog red cells (Parker et al., 1991).

In the 1970s, several *JGP* articles extended the pump-leak idea to include the regulation of the ouabain-insensitive cation "leak" pathways in response to perturbations in cell volume. Kregenow (1971a) used the nucleated erythrocyte of the duck to show that osmotic swelling increases the (ouabain-insensitive) K^+ efflux and causes cell volume to return to normal (Fig. 7 A). Conversely, osmotic shrinkage stimulated ouabain-insensitive Na^+ influx, resulting in cell swelling back toward the normal volume (Kregenow, 1971b; Fig. 7 C). 2 yr later, Parker (1973) showed that the high-Na^+ red cells of the dog are also able to regulate cell volume. The first use of the term "regulatory volume increase" (RVI) was by Cala (1977) in his study of flounder erythrocytes; the same article was the second in PubMed to use the term "regulatory volume decrease" (RVD). The existence of negative feedback systems that adjust ion movements to correct perturbations in cell volume is a central principle of cellular homeostasis.

In the 1980s, articles in *JGP* established the transport processes associated with RVI and RVD in various cells. Cala (1980) showed that RVI and RVD in the large, nucleated erythrocytes of *Amphiuma tridactylum* are mediated by electroneutral transport: Cl^-/HCO_3^- exchange in parallel with Na^+/H^+ exchange (RVI) or K^+/H^+ exchange (RVD). Cala (1983) later demonstrated that Ca^{2+} is a strong modulator of RVI and RVD in *A. tridactylum* red cells. Parker and Castranova (1984) showed that osmotic shrinkage activates Na^+/H^+ exchange in dog red cells. In human lymphocytes, RVD is mediated by K^+ and Cl^- efflux by conductive pathways (Grinstein et al., 1982a,b). If lymphocytes are first swollen osmotically, then allowed to shrink by RVD, and finally shrunken by returning osmolality to normal, the subsequent RVI is mediated by amiloride-sensitive electroneutral Na^+/H^+ exchange (Grinstein et al., 1983), likely as a result of a shrinkage-induced shift in the pK_A of the intracellular H^+ binding event that activates transport (Grinstein et al., 1985).

JGP articles also had an important role in defining the functional and regulatory properties of NKCC (SLC12). Haas et al. (1982) showed that duck erythrocytes exhibit a catecholamine-stimulated, electroneutral, Cl^--dependent 1:1 cotransport

of Na^+ and K^+ and that a Cl^- gradient can drive uphill Na^+ transport (Fig. 7 B), indicating that Cl^- is cotransported with Na^+ and K^+. After NKCCs had been cloned and sequenced, Isenring et al. (1998) used human/shark chimeras as well as point mutagenesis to define the functional roles of residues in specific transmembrane helices in anion transport and bumetanide affinity.

In addition to NKCC, duck erythrocytes also exhibit Na^+-independent cotransport of K^+ and Cl^- (KCC; SLC12), the activity of which is stimulated by cell swelling (Haas and McManus, 1985). Kaji (1986) showed that human red cells also exhibit swelling-activated KCC, and Parker et al. (1991) showed that dog red cells have swelling-activated KCC as well as shrinkage-activated NHE. Several *JGP* articles in the early 1990s concerned the mechanisms by which changes in cell volume activate and inactivate coupled transporters. Jennings and al-Rohil (1990) measured the effects of step increases and decreases in volume on $^{86}Rb^+$ flux in rabbit red cells, which exhibit robust swelling-stimulated KCC. The transporter activates with an exponential time course after a volume increase and inactivates with a more rapid time course after a volume decrease. A two-state model of the regulatory process suggested that the volume-sensitive step is inactivation rather than activation of KCC. The inhibitory effects of okadaic acid suggested further that the volume-sensitive inactivation event is mediated by a protein kinase (Jennings and Schulz, 1991). Parker et al. (1991) observed similar effects in dog red cells (Fig. 7 D), and Dunham et al. (1993) found that a three-state model is needed to explain the kinetics of activation of KCC by cell swelling in low-K^+ sheep red cells.

A very basic question about cell volume regulation is how cells detect volume to initiate transporter-mediated RVI or RVD. The primary cell volume signal was proposed to be the cytoplasmic protein concentration via a macromolecular crowding effect (Colclasure and Parker, 1992; Parker et al., 1995). More recently, the role of kinases and phosphatases in cell volume regulation has remained an area of interest in *JGP*; most of these articles have concerned ion channels rather than exchangers or cotransporters and are beyond the scope of this review.

Neurotransmitter transporters GAT1 (SLC6) and EAAC1 (SLC1)

Cation-coupled cotransporters use the Na^+ gradient to drive the uphill influx of neurotransmitters and remove them from the synaptic cleft. Lu and Hilgemann (1999a) used the excised *Xenopus* oocyte giant patch method in a detailed study of the Na^+ and Cl^--dependent GABA transporter GAT1 (SLC6). As discussed above for other exchangers and cotransporters, the key functional properties of GAT1 are the events in the catalytic cycle, asymmetry, voltage dependence, and secondary modes, all within the framework of the alternating access model. Rapid solution changes made it possible to control substrate concentrations with excellent time resolution (Fig. 8 A). Steady-state transport currents, with systematic variations in the concentrations of all three substrates on both sides of the membrane, showed that GAT1 kinetics are consistent with an alternating-access mechanism rather than the nonstoichiometric channel-like mechanisms that had been proposed by others. Non–steady-state experiments on the same system analyzed how the charge-moving partial reactions depend on substrate concentration (Lu and Hilgemann, 1999b) and provided further support for the alternating access model proposed by Hilgemann and Lu (1999). Li et al. (2000) used fluorescence measurements on voltage-clamped mouse GAT1 expressed in *Xenopus* oocytes to extend this model to include an additional intermediate outward-facing empty state (Fig. 8 B).

Moss et al. (2009), in a *JGP* Tutorial Research Article, used 19 separate constructs of mouse GAT1 fused with fluorescent proteins to investigate oligomerization and trafficking. With pixel-by-pixel measurement of normalized FRET (NFRET; Fig. 8 C), they could quantify FRET intensity in the plasma membrane and perinuclear regions of neuroblastoma 2a cells and detect multiple oligomerization states of GAT1. This article provided new information on GAT1 oligomeric states and demonstrated a powerful technique for studying transporter trafficking and oligomerization.

The excitatory amino acid carrier EAAC1 (SLC 1A1) mediates cation-coupled glutamate uptake in the central nervous system and has a complex catalytic cycle resulting in the influx of 1 glutamate, 2 Na^+, and 1 H^+ and the efflux of 1 K^+ ion. Watzke et al. (2000) used laser-pulse photolysis of caged glutamate to measure pre–steady-state kinetics of EAAC1 and demonstrate that "EAAC1 has to be protonated before glutamate binds at the extracellular side and charge translocation takes place." Watzke et al. (2001) then showed, using photolysis and rapid solution changes, that the extracellular glutamate-binding event is after the first Na^+ and before the third Na^+ binding event. Mim et al. (2005) measured pre–steady-state kinetics of a related glutamate transporter, excitatory amino acid transporter EAAT4, and showed that EAAT4 has kinetics indicating that its role is as a high-affinity, low-capacity transporter that clears the synaptic cleft of low concentrations of glutamate.

Other cation cotransporters

Many cation-coupled cotransporters in addition to NBC, NKCC, KCC, and neurotransmitter transporters have been the subjects of high-impact *JGP* articles. As mentioned above, the Na^+-dependent glucose transporter is an epithelial system and will not be discussed further here (Palmer, 2017). In plants, hexose transport is H^+ coupled; an important early article on H^+-hexose cotransport in *Chlorella vulgaris* thoroughly characterized the kinetics and stoichiometry of the transport (Komor and Tanner, 1974). In yeast (*Saccharomyces cerevisiae*), early work by Armstrong and Rothstein (1967) described the mutual competitive and noncompetitive effects of alkali cations for influx. Rodriguez-Navarro et al. (1986) used electrophysiological recording and rapid solution changes with spherical *Neurospora crassa* cells derived from conidiospores to demonstrate an electrogenic K^+ influx indicative of K^+-H^+ cotransport, which can explain the maximal K^+ gradients generated in *N. crassa* and represented "a new departure in the understanding of cation sequestering in fungi and plants."

Conclusions and future directions

In the past 100 yr, *JGP* has been a premier journal in the field of solute carriers, exchangers, and cotransporters. Thanks in

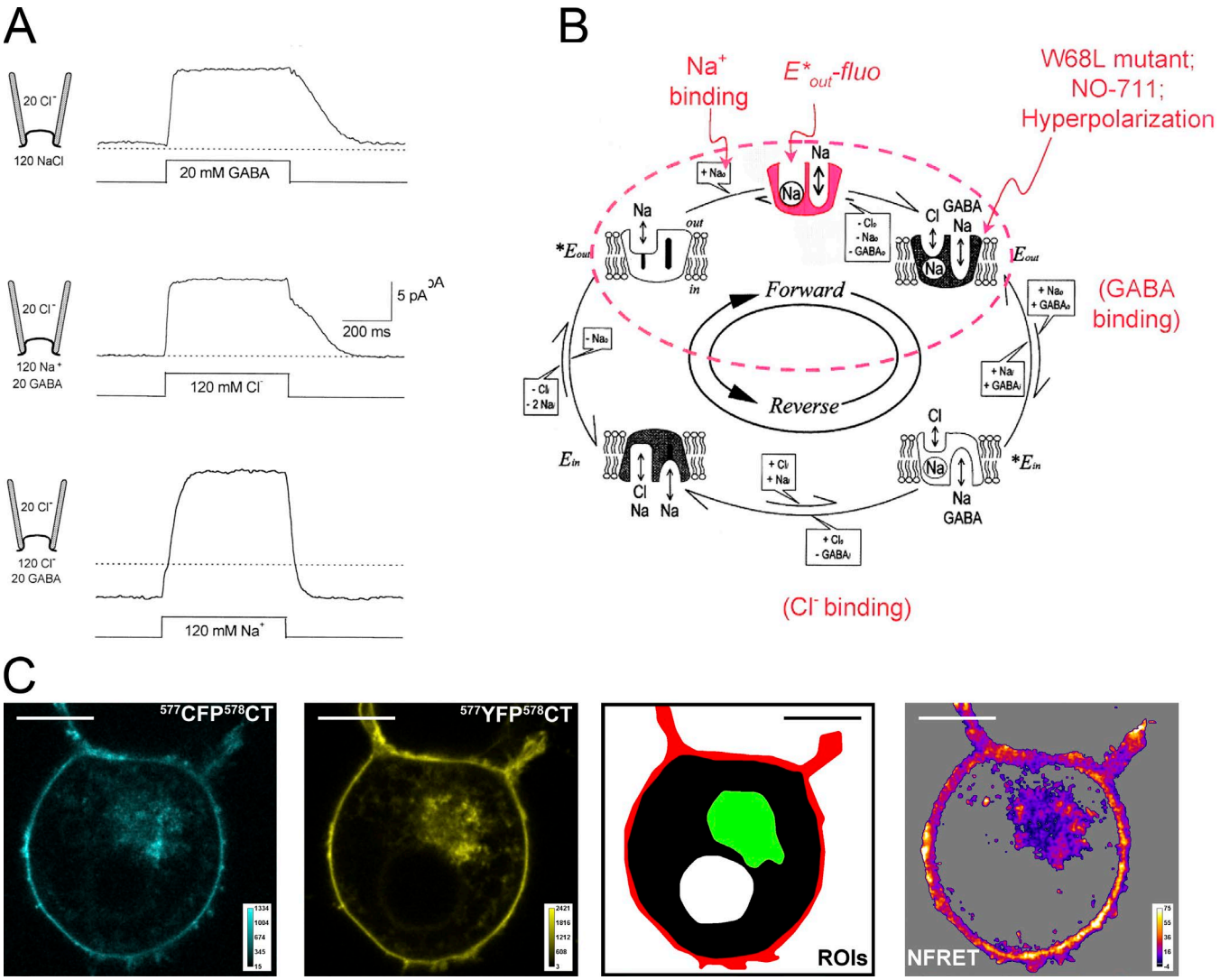

Figure 8. **GABA transporter GAT1. (A)** Rapid solution changes and currents associated with GAT1 in excised oocyte patches (Lu and Hilgemann, 1999a). **(B)** GAT1 catalytic cycle based on model of Hilgemann and Lu (1999), with additional intermediate (red) found by Li et al. (2000). E_{in} and E_{out} are respectively the inward and outward facing states of the transporter. **(C)** NFRET of fluorescent derivatives of GAT1 expressed in neuroblastoma 2a cells (Moss et al., 2009). Bars, 10 µm. First and second panels show fluorescence of GAT1 with C-terminal (CT) fusions with GFP and YFP. Third panel shows definition of regions of interest (ROIs) designated in red (surface) and green (paranuclear). Fourth panel shows NFRET distribution showing high signal in plasma membrane.

part to *JGP* articles, our understanding of solute transporters has progressed from first recognizing their existence 70 yr ago to defining basic functional and regulatory properties, dissecting catalytic cycles and partial reactions, and identifying functionally important amino acid residues. Transporter research in *JGP* encompasses a very wide variety of organisms (bacteria, fungi, plants, invertebrates, amphibians, reptiles, birds, and mammals) and experimental preparations (nerve, heart, skeletal muscle, epithelia, blood cells, cultured cells, expression systems, proteoliposomes, and planar lipid bilayers). Although the biological contexts of transporter articles in *JGP* have been quite varied, a common thread is a quantitative, mechanistic approach, often involving mathematical derivations. Another common thread is careful control of experimental conditions on both sides of the membrane, reflecting the understanding that meaningful interpretation of transport measurements requires knowledge of driving forces. *JGP* transporter articles have often combined different technologies (e.g., electrophysiology, tracers, and optical methods) and have set new standards for time resolution for both data collection and step changes in conditions. Recent structure-based *JGP* articles are moving toward a molecular-level understanding of carriers, exchangers, and cotransporters.

What does the future hold for transporter research in *JGP*? Computational modeling based on structures will likely be an increasingly important approach for studying transporters. For example, Vergara-Jaque et al. (2015) used a computational approach to examine the structural similarities among three bacterial coupled transporters and demonstrated a remarkably similar fold and similar amino acid motifs in the substrate-binding site despite the diverse functions of the three transporters. In addition to structural approaches, *JGP* articles on transporters will continue to use state of the art technologies in electrophysiology, cellular imaging, kinetic modeling, and platforms for reconsti-

tuted membrane proteins. The scientific questions will continue to include molecular mechanisms of action and regulation of individual transporters but will likely expand to include more integrative studies on how transporters, together with pumps, channels, metabolism, and signaling pathways, regulate cell, tissue, and whole-organism development and homeostasis. There is more to be learned about transporters, and much of the new knowledge will very likely be published in *JGP*.

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Olaf S. Andersen served as editor.

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