

REVIEW ARTICLE

Regulation of intracellular pH in eukaryotic cells

Inger Helene MADSHUS

Department of Biochemistry, Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, 0310 Oslo 3, Norway

INTRODUCTION

The fact that cytoplasmic pH is strictly regulated has only been appreciated during the last 10 years. Eukaryotic cells clamp cytoplasmic pH at 7.0–7.4 by ion transport mechanisms and a high buffering capacity of the cytosol. The values of internal pH observed (pH 7.0–7.4) are higher than expected if the protons had been passively distributed across the cell membrane according to electrochemical gradients. Thus, when the membrane potential is -59 mV, pH_i should be 1 unit less than pH_o . The equilibrium relation between membrane potential, V_m , and internal and external H^+ concentrations is given by the Nernst expression:

$$V_m = 1000 (R \times T/F) \ln [H^+]_o/[H^+]_i$$

where R and F are the gas constant and Faraday constant respectively, and V_m is membrane voltage in mV. At 22°C , $V_m = 59$ ($pH_i - pH_o$). At extracellular pH 7.4 the calculations would predict the cytosolic pH to be 6.4. This pH value is cytotoxic and far below the one actually observed. In barnacle muscle pH_i was measured to be 0.5 unit higher than expected from the electrochemical gradient (Roos & Boron, 1982), and similar findings have been made with a number of cell types. This fact clearly shows that there are mechanisms actively removing acid equivalents from the cytosol.

The buffering capacity of cells has been determined to be between 10 and 50 mM per pH unit, depending on cell type investigated and on the conditions of the measurements (whether or not the buffering capacity has been measured in the presence of bicarbonate).

Because of the great importance of the internal pH for many cellular processes and because the field concerning pH-regulating mechanisms is rapidly expanding, I here present an updated overview of the reasons why intracellular pH must be strictly controlled, the methods used to study regulation of intracellular pH and the mechanisms involved in the regulation of intracellular pH.

REASONS WHY INTRACELLULAR pH MUST BE STRICTLY CONTROLLED

Intracellular pH is important for the activity of a number of enzymes with pH optima within the physiological pH range as well as for the efficiency of contractile elements and the conductivity of ion channels. Also, pH oscillations seem to be important in controlling the cell cycle and the proliferative capacity of cells.

Effect of pH on the activity of metabolic enzymes and synthesis of macromolecules

The activity of a large number of intracellular enzymes taking part in the cellular metabolism is pH-sensitive. An

important example is phosphofructokinase, the rate-limiting enzyme of glycolysis. The activity of this enzyme strongly increases with increasing pH over a small pH interval within the physiological range (Fidelman *et al.*, 1982; Ui, 1966). Insulin stimulates the key enzyme of the glycolysis by increasing the pH_i by activating the electroneutral Na^+/H^+ -exchanger in the plasma membrane (Moore, 1981). In agreement with the results described above, Seglen (1972) reported that in perfused rat liver cells both glycolysis and respiratory activity were inhibited by low pH.

Also, protein synthesis is affected by pH. Thus, in a cell-free translation system Winkler (1982) found a sharp increase in the rate of protein synthesis starting at pH 6.9 with an optimum at pH 7.4.

The synthesis of DNA and RNA increase with increasing intracellular pH within the physiological range. The pH optimum of DNA polymerases is generally quite high. The activity increases with increasing pH from 7.0 to 8.0, which encompasses the usual physiological pH_i range (Gerson, 1982). This can probably be related to the rise in the free energy of hydrolysis of ATP and other nucleoside triphosphates observed with increasing pH (Alberty, 1968).

Within the physiological range, it appears to be a general rule that with increasing pH_i the metabolic activities of cells increase.

Effect of pH on contractile elements

The contractile activity of purified preparations of actin and myosin has been shown to be dramatically influenced by comparatively small changes in pH (Condeelis & Taylor, 1977) with low pH reducing the contractility. Also, microtubule assembly and disassembly is affected by pH with an increased disassembly at alkaline pH (Regula *et al.*, 1981).

Acidification dramatically reduces the contractility of muscles. Apparently, intracellular acidosis may account for between 40% and 50% of the immediate negative inotropic effect of ischaemia in the heart muscle (Jacobus *et al.*, 1982).

Effect of pH on ion conductivities

Some ion channels have a pH-dependent conductivity. In particular, potassium channels in excitable cells are often pH-dependent (see Moody, 1984). Intracellular acidification blocks the K^+ conductance and depolarizes the membrane, thereby facilitating the occurrence of action potentials. pH-sensitive K^+ channels have been shown to be of importance for the generation of Ca^{2+} -dependent action potentials in crayfish slow muscle fibres (Moody, 1982). Also, in the case of vertebrate muscle fibres (Blatz, 1980) and in the squid giant axon (Wanke *et al.*, 1979) the K^+ conductance was shown to decrease with increasing intracellular acidification.

Changes in ionic conductance in the pancreatic β -cell plasma membrane most likely represent fundamental steps in stimulus-secretion coupling. The glucose-induced electrical activity in β -cells has been shown to be modulated by pH_i through the effect on K^+ channels (Tarvin *et al.*, 1981; Rosario & Rojas, 1986). When the K^+ -channel conductance decreases, the membrane is depolarized and voltage-gated Ca^{2+} channels and Na^+ channels are activated (Pace *et al.*, 1982). The resulting increase in Ca^{2+}_i is a stimulus for exocytosis of insulin-containing vesicles. pH_i changes thereby indirectly regulate the release of insulin.

With respect to Ca^{2+} channels, Umbach (1982) found that in *Paramecium* the Ca^{2+} currents were decreased by decreasing pH_i . The titration effect indicated a single titratable group with an apparent dissociation constant of 6.2. In *Aplysia* neurons, however, no effect on Ca^{2+} currents was observed when pH_i increased by 0.35 unit (Zucker, 1981). However, the resting pH_i in *Aplysia* neurons is 7.17, as opposed to 6.8 in *Paramecium*. The titration curve obtained by Umbach predicts that no effect on the Ca^{2+} currents is obtained when pH_i is raised to values above 7.17. In contrast with K^+ channels, comparatively little is known about the pH-dependency of Ca^{2+} channels. A number of workers have measured the effects of an imposed pH_i change on Ca^{2+}_i . However, the effect on Ca^{2+}_i of changing pH_i is not uniform among cells and cannot be predicted with confidence (see Moody, 1984).

In *Xenopus* embryos the conductance of gap junctions was found to be blocked by decreasing pH (Turin & Warner, 1977, 1980). The conductance changed steeply over a small pH_i interval. In experiments on isolated coupled cells in which the pH_i and the junctional conductance were directly measured, the relationship was shown to be a simple sigmoid curve (Spray *et al.*, 1982). The relationship was well fitted by a Hill plot with an apparent pK_H of 7.3.

This suggested that pH_i changes might play a physiological role in modifying electrical communications between cells (Spray *et al.*, 1982).

Altogether, it appears that changes in pH_i modify the electrical properties of excitable cells. Thus, small pH_i changes dramatically influence the responsiveness of such cells (Moody, 1984). Also, intercellular communication via gap junctions, which is important in development and in organized functioning of tissues, is highly sensitive to pH changes (Spray *et al.*, 1982).

Control of the cell cycle

Oscillations in intracellular pH have been postulated to be of importance in the control of the cell cycle and cell division in several cell types. Low intracellular pH is common both to prokaryotic and eukaryotic resting cells. This is one reason that these cells have low metabolic activities. A rapid increase in intracellular pH may be important to bring cells from G_0/G_1 and into S phase.

Many observations exist with respect to pH oscillations during the cell cycle. In the ciliated protozoan *Tetrahymena pyriformis* two pH increases of about 0.3–0.4 pH unit above a baseline of 7.2 were observed within 30 min of the onset of S phase (Gillies & Deamer, 1979). A single pH_i shift of similar magnitude has been reported in the slime mould *Physarum polycephalum* (Gerson & Burton, 1977). In this case the pH_i increase was associated with

cell division and mitosis. These authors suggest that pH_i increases play a significant role in induction of cell proliferation and cell division.

Steinhardt & Morisawa (1982) also presented evidence for a pH-sensitive step prior to mitosis in *Physarum polycephalum*. In the cell cycle the pH_i was increased to pH 7.4–7.5 within 1 h prior to mitosis. If pH_i was artificially lowered, a delay of mitosis was induced. Furthermore, starvation of the cells lowered the pH_i and interrupted the cell cycle, thereby inhibiting mitosis.

According to Gerson (1982) a biphasic increase in pH_i was observed when lymphocytes were stimulated mitogenically with concanavalin A. The first peak was seen after 6–8 h and the second peak 48 h after the stimulation. The first peak correlated with early events, such as increase in the synthesis of phospholipids and protein, while the second peak correlated with the synthesis of DNA.

The results described above support the hypothesis that intracellular pH is an important modulator of cell proliferation and cell division. Experiments with early *Xenopus* embryos indicated, however, that pH_i is not necessarily a universal regulator of mitosis. It was reported that even if small oscillations in pH_i occurred during the mitotic cycle in early *Xenopus* embryos, no delay in mitosis occurred when the pH_i was artificially reduced (Lee & Steinhardt, 1981). Furthermore, clamping at high pH_i values did not interfere with normal chromosome cycling or cell division in the sea urchin embryo (Grainger *et al.*, 1979). This suggests that the pH_i shifts observed in other systems (*Tetrahymena* and *Physarum*) reflect changes associated with cell growth but not mitosis or cytokinesis *per se*.

Pouyssegur *et al.* (1985) reported that in response to growth factors, quiescent fibroblast mutants lacking Na^+/H^+ exchange activity failed to elevate their cytoplasmic pH and to reinitiate DNA synthesis at neutral and acidic pH_0 . These authors claim that a pH_i threshold of 7.2 exists, below which growth factors cannot induce the G_0 to S phase transition.

In contrast with this, Mills *et al.* (1985) suggested that pH_i increase is not essential for proliferation. This conclusion was based upon observations with interleukin 2-induced lymphocyte proliferation. Interleukin 2 stimulates electroneutral Na^+/H^+ exchange, thereby increasing pH_i . However, if this exchanger was inhibited with amiloride analogues in a HCO_3^- -free medium, proliferation occurred even if pH_i did not increase.

A principal difference in the experimental design between the experiments of Pouyssegur *et al.* (1985) and Mills *et al.* (1985) could explain why these authors reached different conclusions. Lymphocytes having interleukin 2 receptors are in G_1 phase; this means that the cells are partly stimulated and thereby already in the cell cycle, while quiescent fibroblasts are in G_0 and not in the cell cycle. Possibly, a pH_i increase is necessary for the transition of cells from G_0 to G_1 in order to bring quiescent cells back into the cell cycle.

It was recently reported by Ober & Pardee (1987) that a series of tumorigenic Chinese hamster embryo fibroblast cell lines maintain an internal pH that is 0.12 ± 0.04 pH unit above that of the non-tumorigenic parental cell line. This suggests a critical role for pH_i in the regulation of DNA synthesis and suggests that aberrations in pH_i can contribute to the acquisition of altered growth properties.

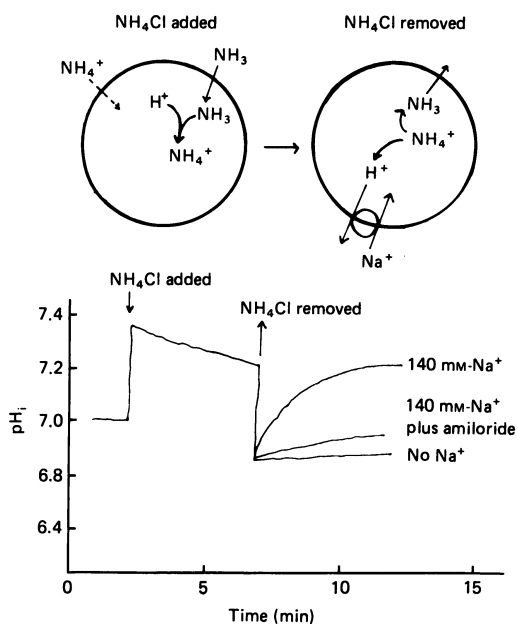


Fig. 1. Na^+/H^+ antiport studied by the ammonium prepulse technique

When the prepulse is applied, an abrupt acidification of the cytosol is produced. This acidification is followed by a rapid pH_i normalization in a sodium-containing buffer due to Na^+/H^+ antiport. The normalization follows an exponential time-course, being complete after 5 min in a bicarbonate-free medium.

Altogether, most data presented support the hypothesis that a strictly controlled pH_i acts as a second messenger in growth control.

METHODS TO STUDY INTRACELLULAR pH REGULATION

A number of different techniques are now available to measure intracellular pH. For a detailed description of these methods the reader is referred to Nuccitelli & Deamer (1982). Here the different methods are only mentioned briefly.

A widely used method for estimating pH_i is to measure the equilibrium distribution of a radioactively labelled weak acid or base across the plasma membrane. However, this method has limitations such as sensitivity to cellular volume changes and intracellular compartmentalization of the tracer (Roos & Boron, 1981). Also, this method shows a poor temporal resolution.

N.m.r. was first used to measure intracellular pH in red blood cells by Moon & Richards (1973). The inorganic phosphate signal is most commonly used, because it is readily observable in the majority of ^{31}P spectra and because its frequency is particularly sensitive to pH changes in the region around neutrality (Gadian *et al.*, 1982). A disadvantage is that n.m.r. equipment is expensive and complicated to use. The requirement for a high cell density also creates problems, but these can be solved by various methods of superfusion (Nuccitelli, 1982).

pH-sensitive microelectrodes offer one way of obtaining continuous pH measurements. The recessed-tip type of pH microelectrode, first described in 1974 by Thomas, can be made with tip diameters of less than $1\ \mu\text{m}$, and can thus be used on a wide variety of cells. However, one is at risk of destroying the cell when the electrode is introduced through the cell membrane.

When regulatory mechanisms are studied, it is convenient to use fluorescent probes with pH-dependent fluorescence. Most often carboxylfluorescein derivatives are used. For these compounds the excitation, and thereby the fluorescence intensity, increases almost linearly from pH 6.5 to pH 8.0. Fluorescent probes can be introduced to the cytosol in the form of lipid-soluble esters, which are cleaved by cytoplasmic esterases, and the fluorophore is thereby trapped in the cytosol (Thomas *et al.*, 1979; Rink *et al.*, 1982; Moolenaar *et al.*, 1983). Such techniques are non-invasive, and spectrally monitored fluorescence can be recorded continuously with excellent temporal resolution.

To study pH regulation the cytosol may be acidified or alkalinized and then the regulation of the pH back to normal value is studied under different conditions by a continuous recording of pH. In this way different mechanisms for pH regulation have been characterized, and, in the following, four such mechanisms will be described in detail.

MECHANISMS INVOLVED IN THE REGULATION OF INTRACELLULAR pH

Na^+/H^+ antiport

The first demonstrations of Na^+/H^+ antiport in eukaryotic plasma membranes were made in vesicles from the brush borders of rabbit kidney and small intestine (Murer *et al.*, 1976). Electroneutral Na^+/H^+ antiport was described as an important pH-regulating mechanism in sheep heart Purkinje cells after cytoplasmic acidification (Deitmer & Ellis, 1980). Recently, much information has accumulated regarding the Na^+/H^+ exchanger in mammalian cells, and there seems to be general agreement that all animal cells possess an electroneutral Na^+/H^+ antiporter (for review, see Krulwich, 1983). The antiporter responds to a fall in extracellular pH by quickly extruding protons in exchange with extracellular Na^+ (Fig. 1). The energy for the extrusion is provided by the large inward-directed Na^+ gradient.

The cytosol can be acidified by the ammonium prepulse technique described by Boron & De Weer (1976). Addition of NH_4Cl to the culture medium first produces a rapid rise in pH_i due to entry of NH_3 , which combines with protons in the cytosol. The pH_i subsequently slowly decays due to entry of NH_4^+ . When NH_4Cl is withdrawn from the medium, NH_3 leaves the cells while H^+ ions are left behind. Therefore, the pH becomes lower than the original pH_i value before the pulse was applied (Fig. 1).

The Na^+/H^+ antiporter is inhibited by the potassium-saving diuretic amiloride and by analogues of this drug. Amiloride inhibits competitively binding to the extracellular site which accommodates Na^+ ions (Haggerty *et al.*, 1985).

During extrusion of protons after an acid load, a large amount of Na^+ ions enter the cell due to Na^+/H^+ exchange. The Na^+ ions are then extruded into the

medium by the Na^+/K^+ -ATPase. If the ATPase is inhibited with ouabain, the Na^+/H^+ antiporter will eventually be inhibited, because the sodium gradient, which drives the process, is dissipated. Depending on the direction of the Na^+ gradient, H^+ ions will either enter or leave the cell by Na^+/H^+ exchange. At low extracellular Na^+ the sodium gradient is reversed, and cytoplasmic acidification will follow when the Na^+/H^+ exchanger is stimulated (Moolenaar *et al.*, 1983; Paris & Pouyssegur, 1983).

Normally, the rate of Na^+/H^+ exchange is small, just balancing the passive H^+ influx and the intracellular production of acidic metabolites. The Na^+/H^+ exchanger is *regulated*; otherwise the actual Na^+ gradient would mediate an H^+ efflux, resulting in a resting pH_i more alkaline than the actual pH_i (Moolenaar, 1986).

There is evidence that the intracellular pH is the important parameter regulating the activity of the exchanger. Aronson and coworkers proposed that cytoplasmic H^+ acts as an allosteric activator of the Na^+/H^+ exchanger (Aronson *et al.*, 1982). It has been suggested that the antiporter molecule has on its cytoplasmic face two proton-binding sites that are separate and functionally independent. One site is a regulatory or modifier site. When this site is occupied, a conformational change is triggered, activating the exchanger. A distinct H^+ -transport site mediates the net extrusion of H^+ once the exchanger is activated (Aronson *et al.*, 1982; Aronson, 1985).

The amiloride-sensitive Na^+/H^+ exchanger seems to be involved in hormonal stimulation of cell growth (Smith & Rozengurt, 1978; Moolenaar *et al.*, 1982; Schulinder & Rozengurt, 1982). There is evidence that mitogen-induced cytoplasmic alkalization is due to Na^+/H^+ exchange (Rothenberg *et al.*, 1982, 1983a,b; Cassel *et al.*, 1983; Moolenaar *et al.*, 1982, 1983; L'Allemain *et al.*, 1984). Experiments indicate that serum in human fibroblasts (Moolenaar *et al.*, 1983), thrombin in hamster fibroblasts (Paris & Pouyssegur, 1983) and phorbol esters in T-lymphocytes (Grinstein *et al.*, 1985) may activate the Na^+/H^+ antiporter by increasing the affinity of the pH_i sensor, the allosteric H^+ -binding site.

The understanding of the sequence of events mediating the growth-factor-induced rise in pH_i is still incomplete. Protein kinase C is a strong candidate for a transducer of growth-factor-mediated activation of the Na^+/H^+ antiporter. The fact that a variety of extracellular signals stimulate inositol phospholipid breakdown and therefore the formation of endogenous diacylglycerol supports this possibility (Nishizuka, 1984; Berridge, 1984). Also, phorbol 12-myristate 13-acetate and other phorbol esters were suggested to stimulate Na^+/H^+ exchange through an effect on protein kinase C (Grinstein *et al.*, 1985). However, the activation of the antiporter by growth factors may also be mediated by other pathways, such as steps activated by Ca^{2+} -calmodulin or GTP-binding proteins. Also, transmethylation reactions have been proposed to modulate the activity of the Na^+/H^+ -exchanger (Dudeja *et al.*, 1986).

It can be concluded that the Na^+/H^+ antiporter is apparently present in all animal cells and that it plays an important role in regulation of pH_i back to normal value after an acid load. Also, the antiporter seems to mediate an alkalization of the cytosol when hormones and growth factors combine with their cellular receptors.

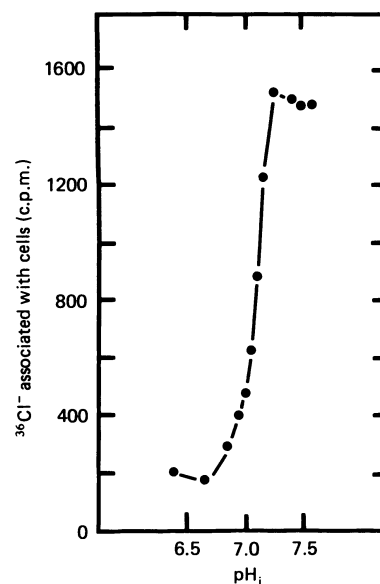


Fig. 2. Effect of intracellular pH on the uptake of $^{36}\text{Cl}^-$ by anion antiport in Vero cells

For experimental details, see Olsnes *et al.* (1987b).

Anion antiport

Most cells require bicarbonate for continuous growth. One reason for this may be that $\text{Cl}^-/\text{HCO}_3^-$ antiport is important in the regulation of pH_i . A bicarbonate-linked mechanism was suggested by Russell & Boron (1976) to be the most important pH regulatory mechanism in invertebrate cells.

The earliest experiments studying pH_i regulation were performed on large invertebrate cells such as snail neurons and squid axons. The registrations of intracellular pH were continuous by pH-sensitive microelectrodes applied intracellularly. Thomas (1982) reported that in snail neurons intracellular chloride and extracellular HCO_3^- were necessary to normalize pH_i after an acid load. He also found the system to be electroneutral and dependent on extracellular Na^+ . Furthermore, the pH regulation was completely inhibited by the anion-exchange inhibitor SITS. Becker & Duhm (1978) proposed that pH_i might be regulated by an exchange of internal Cl^- for an external ion pair consisting of one Na^+ ion linked to one CO_3^{2-} ion.

$\text{Cl}^-/\text{HCO}_3^-$ exchange has been described to be Na^+ -dependent in hamster fibroblasts (L'Allemain *et al.*, 1985), and in squid axon (Boron, 1985), while Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchange has been described in sheep heart Purkinje fibres (Vaughan-Jones, 1979), and in LLC-PK₁ cells (Chaillet *et al.*, 1986). Evidence was recently presented that both Na^+ -dependent and Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ antiport take place in Vero cells (Tønnessen *et al.*, 1987; Olsnes *et al.*, 1987a; Madshus & Olsnes, 1987).

It was suggested by Russell & Boron (1976) that the squid axon might possess an ATP-dependent pump which responded to acid challenges by taking up HCO_3^- from the medium in exchange with intracellular chloride. However, convincing evidence for the existence of an energy-dependent mechanism involved in anion

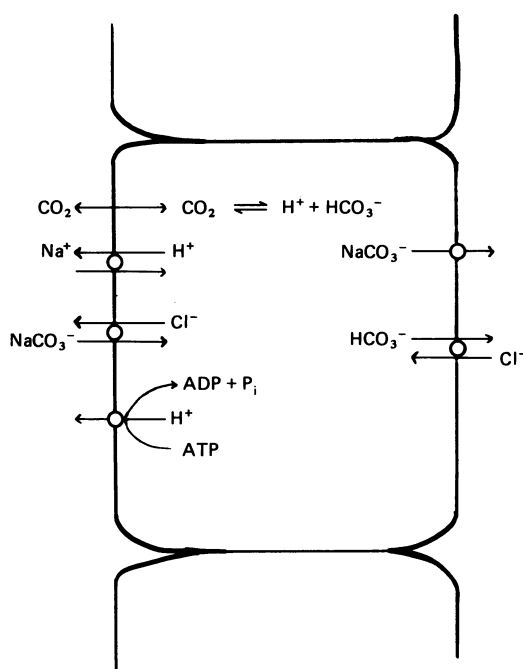


Fig. 3. Schematic drawing showing pH-regulatory mechanisms described in the text

On the left side mechanisms correcting acidification of the cytosol are depicted, while on the right side pH-regulatory mechanisms producing intracellular acidification or correcting intracellular alkalization are depicted.

antiport has not been presented. The energy available from the large inward-directed Na⁺ gradient is more than sufficient to drive the uptake of NaCO₃⁻ and the efflux of Cl⁻. Cl⁻ and HCO₃⁻ ions are relatively close to electrochemical equilibrium.

It can be concluded that cytoplasmic pH can be effectively normalized after an acid load by anion antiport. HCO₃⁻/Na⁺ as a negatively charged complex is exchanged with intracellular chloride, and the process is probably driven by the sodium gradient and in some cases by an additional inward-directed HCO₃⁻ gradient.

The ion gradients are such that the Na⁺-independent Cl⁻/HCO₃⁻ exchanger will under physiological conditions bring chloride ions into the cell and bicarbonate out of the cell. Thus, the [Cl⁻]_o/[Cl⁻]_i is generally greater than [HCO₃⁻]_o/[HCO₃⁻]_i and a passive and electrically neutral Cl⁻/HCO₃⁻ exchanger would therefore normally mediate Cl⁻ uptake and HCO₃⁻ extrusion. This process could be of importance in keeping the Cl⁻ activity above the electrochemical equilibrium value in certain cell types (Vaughan-Jones, 1979). Because of the HCO₃⁻ efflux this antiport would with time result in an acidification, which would have to be compensated for by another pH regulatory mechanism.

The Na⁺-independent Cl⁻/HCO₃⁻ exchanger may be of importance in situations where the pH_i rises above neutrality. pH_i regulation on the alkaline side of the normal pH_i is of interest because both the coupled NaCO₃⁻/Cl⁻ exchange and the Na⁺/H⁺ exchange regulate pH_i only on the acid side. It was shown by Olsnes

et al. (1986) that the Na⁺-independent anion antiporter shows a steep rise in V_{max} with increasing pH_i above pH 7.0. In Fig. 2 is shown how ³⁶Cl⁻ uptake by anion antiport increases strongly over a narrow pH interval.

An electroneutral Cl⁻/HCO₃⁻ exchanger could also participate in acid extrusion, but only if pH_i falls sufficiently to raise [HCO₃⁻]_o/[HCO₃⁻]_i above [Cl⁻]_o/[Cl⁻]_i. Also, if the chloride gradient was reversed by removing extracellular chloride the sodium-independent anion antiporter was shown to extrude acid equivalents (Tønnessen *et al.*, 1987; Madshus & Olsnes, 1987).

Compared with the great amount of information accumulating regarding the regulation of Na⁺/H⁺ antiport, there has been little information regarding the regulation of the anion antiport. Possibly, the activity of the sodium-linked and the sodium-independent Cl⁻/HCO₃⁻ antiporters is subject to regulation by hormones and growth factors, in a similar way as the Na⁺/H⁺ antiporter.

It can be concluded that anion antiport mechanisms constitute important pH regulatory mechanisms and that by anion antiport pH_i can be normalized both after acid and alkali loads. After an acid load, pH_i is normalized by the uptake of a charged complex of Na⁺ and HCO₃⁻ in exchange with intracellular Cl⁻, while after an alkali load pH_i is normalized by extrusion of intracellular HCO₃⁻ in exchange with extracellular Cl⁻. It is still unclear whether different molecular entities are involved in the two kinds of anion antiport.

Na⁺/HCO₃⁻ symport

A number of authors have recently presented evidence for electrogenic symport of Na⁺ and HCO₃⁻ in a variety of cells involved in transepithelial transport of acid (Boron & Boulpaep, 1983; Alpern, 1985; Jentsch *et al.*, 1985, 1986b; Yoshitomi *et al.*, 1985; Biagi & Sohtell, 1986). This symport has been described to couple tightly the transport of one Na⁺ ion to two or three HCO₃⁻ ions. It has also been suggested that the negatively charged complex is the ion pair NaCO₃⁻ (Jentsch *et al.*, 1986a). The transport is inhibited by the anion exchange inhibitors SITS and DIDS. The first example of such transport was described to take place in the basolateral membrane of the proximal tubule of the salamander *Ambystoma tigrinum* (Boron & Boulpaep, 1983). The transporter was postulated to mediate net efflux of HCO₃⁻ and Na⁺, giving rise to transcellular transport of HCO₃⁻. A similar process was described in monkey kidney epithelial cells (Jentsch *et al.*, 1985), and in proximal tubular cells of the rat (Alpern, 1985; Yoshitomi *et al.*, 1985) and the rabbit (Biagi & Sohtell, 1986; Sasaki *et al.*, 1985).

In all these cases it was found that a sudden peritubular reduction of Na⁺- and/or HCO₃⁻ concentration caused a sudden transient depolarization as well as a reduction in pH_i. The cytoplasmic acidification indicates that bicarbonate efflux takes place in response to peritubular reduction of bicarbonate and sodium ions. The fact that bicarbonate efflux takes place in response to an altered sodium gradient indicates strongly that a complex of Na⁺ and HCO₃⁻ is transported, and the resulting depolarization indicates that this transport is electrogenic.

The 'leakage' of HCO₃⁻ across the basolateral side of the polarized cell membrane mediates intracellular

acidification and must be compensated for by H^+ extrusion across the luminal membrane. Such H^+ extrusion has been shown to occur by the Na^+/H^+ antiporter and by H^+ -translocating ATPases (see below). The H^+ extruded from the cell across the luminal membrane constitutes transcellular acid secretion. Thus, acid secretion is merely a byproduct of pH_i regulation; the efflux of HCO_3^- across the basolateral membrane provides a sustained intracellular acid load to which the Na^+/H^+ exchanger and the electrogenic H^+ -translocating ATPase respond by extruding protons across the luminal membrane (see Boron, 1983).

The possibility exists that some of the transport phenomena described as Na^+/HCO_3^- symport actually partly consists of a $Cl^-/NaCO_3^-$ antiport. It seems that the Cl^- independence for the transport has not been fully documented in all cases. $Cl^-/NaCO_3^-$ antiport has been described to be an important mechanism in pH_i normalization after an acid load. The possibility exists that this antiporter could function in both directions depending on the electrochemical ion gradients.

In the case of the Cl^-/HCO_3^- antiport and Cl^-/Cl^- antiport, a certain slippage has been shown to take place at alkaline pH values in the sense that the coupling ratio between the anions is different from 1 (Olsnes & Sandvig, 1986; Olsnes *et al.*, 1987b). The same could be true for the $Cl^-/NaCO_3^-$ antiporter; at certain conditions more or less slippage and thereby electrogenic transport could occur.

Na^+/HCO_3^- symport mechanisms have been described almost exclusively in cells from renal tubules. These cells are specialized in the sense that they have the important function of transcellular transport of acid. Such cells have high concentrations of the enzyme carbonic anhydrase. It is still not clear whether Na^+/HCO_3^- symport constitutes a general pH_i regulatory mechanism or if this symport is only operating in specialized acid-secreting cells.

H^+ -translocating ATPases

Proton pumps driven by hydrolysis of ATP have been described in the plasma membrane of certain tight epithelia, such as turtle urinary bladder (Steinmetz, 1974; Gluck *et al.*, 1982). Proton-translocating ATPases have also been described in a number of intracellular organelles (Anderson *et al.*, 1982; Hutton, 1982; Dean *et al.*, 1984; Forgac *et al.*, 1983; Galloway *et al.*, 1983; Glickman *et al.*, 1983). Intracellular vesicles with proton pumps fuse with and form from the plasma membrane, and it has been suggested that H^+ pumps may participate in cellular pH regulation (Adelsberg & Al-Awqati, 1986).

Proton pumps are electrogenic, but are either coupled in parallel with anion channels or antiparallel to K^+ channels in order to counteract the buildup of a membrane potential (for review, see Mellman *et al.*, 1986).

Even if the proton pumps are not located at the plasma membrane they can actively remove H^+ ions from the cytosol.

The pH of intracellular vesicles has been shown to be between 4.5 and 6, with the lowest pH values found in lysosomes (see Mellman *et al.*, 1986).

When Hep 2 cells were treated with a hypo-osmotic buffer, an immediate cytoplasmic acidification of approx.

0.2 pH units was observed simultaneously with an alkalization of the vesicular compartment (Madshus *et al.*, 1987). This suggests that swelling of intracellular vesicles has taken place with resulting leakage of intravesicular protons to the cytosol and indicates that the vesicular compartment normally is of great importance in removing H^+ ions from the cytosol.

Secretion of acid in renal proximal tubular cells is suggested to be partly mediated by proton-translocating ATPases (Steinmetz & Andersen, 1982). The acid secretion is a two-step process. Acid first enters the cell across the basolateral membrane by HCO_3^- efflux (see Na^+/HCO_3^- symport) and acid then exits the cell across the luminal membrane, by luminal H^+ pumps and by Na^+/H^+ antiporters in the luminal membrane.

Cannon *et al.* (1985) reported that the number of H^+ -ATPases in the apical plasma membrane of turtle-bladder cells was regulated by exocytic insertion of intracellular vesicles whose membrane contained the H^+ pump. CO_2 , which is a major stimulus for urinary acidification, was shown to cause rapid fusion of these vesicles with the luminal membrane, thereby inserting these pumps and increasing the rate of net transepithelial H^+ secretion. Exocytosis was also shown to take place when intracellular pH was decreased by incubation of the cells with weak acids (Cannon *et al.*, 1985). These results suggest that rapid insertion of proton pumps may be an important mechanism of cell pH regulation in addition to other previously described mechanisms.

Altogether, it appears that proton-translocating ATPases are important in expelling protons from the cytosol both when the pumps are located in the plasma membrane and when located in the limiting membrane of intracellular vesicles.

CONCLUDING REMARKS

Most eukaryotic cells appear to have two or more pH-regulating mechanisms and the interaction between the different mechanisms is complicated. The pH-regulatory mechanisms described above are schematically drawn in Fig. 3. There is a general agreement that all cells possess an electroneutral Na^+/H^+ antiporter and that this antiporter is important for the regulation of pH_i after acid loads. The Na^+/H^+ antiporter also seems to be important for the elevation of pH_i by hormones and growth factors.

Most cells possess pH-regulating anion antiport mechanisms. By way of anion antiport, cells can normalize pH_i after acid and alkali loads. Both cation and anion antiport mechanisms thus are important in the regulation of pH_i after acid loads. The relative importance of these mechanisms probably varies between different cells.

Whether Na^+/HCO_3^- symport is a general pH-regulatory mechanism or if such transport only occurs in specialized cells important for transcellular transport of acid is still unclear. Finally, it should be noted that proton pumps efficiently remove H^+ ions from the cytosol both when located in the membrane of intracellular vacuoles and when located in the plasma membrane.

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