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*Topical Review*

# Ion Concentration-Dependent Regulation of Na,K-Pump Abundance

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## Introduction

The ability of living cells to respond to changes in their environment may be one of their most fundamental properties. In response to an external perturbation, cells will adjust various intracellular processes so as to maintain overall homeostasis. For example, the cells of euryhaline invertebrates must adapt to the changing osmotic pressures imposed by variations in salinity. To remain isomotic with the environment, they regulate the intracellular concentrations of simple organic solutes such as glycine or taurine. In this way, these organisms avoid large perturbations in the intracellular concentrations of the major ions (for a review, *see* Gilles, 1979). An additional example of a cellular response to environmental change is provided by *Escherichia coli*. Changes in the availability of important nutrients to the bacterium often result in the modulation of many catabolic and biosynthetic pathways, such as lactose utilization and tryptophan synthesis. Indeed, this regulation has been the subject of many now-classical studies of prokaryotic gene regulation and induction (e.g., Jacob & Monod, 1961).

This acclimation to environmental change is not limited to invertebrates and free-living cells. Mammalian cells possess the ability to compensate for a decrease in the interstitial concentration of some important solutes and nutrients. These homeostatic mechanisms may be rarely invoked except under pathological conditions, but their presence can be demonstrated in vitro. Exposure of cultured cells to decreased concentrations of various nutrients often results in an enhanced transport of those nutrients. For example, exposure of chick and human fibroblasts to glucose-depleted medium evokes an increased capacity for $Na^+$-dependent hexose uptake (Kletzien & Perdue, 1975; Salter & Cook, 1976). A similar increase in amino acid transport is produced by the exposure of HeLa and rat hepatoma cells to reduced concentrations of amino acids (Hume & Lamb, 1974; Heaton & Gelehrter, 1977).

Because of its role as the major intracellular cation, changes in the extracellular concentration of $K^+$ evoke one of the more important of these cellular responses to environmental change. In a number of cell lines, a decreased concentration of $K^+$ in the culture medium results in an increased capacity for absorption of $K^+$ and extrusion of $Na^+$. This enhanced transport is mediated by the Na,K pump, the protein complex responsible for generation and maintenance of the transmembrane gradients for these cations (Boardman, Lamb & McCall, 1972; Vaughan & Cook, 1972; Boardman et al., 1974). The regulatory events that take place in response to low external concentrations of $K^+$ may be divided into two temporal domains, as suggested by Cook and his colleagues for HeLa cells (Pollack, Tate & Cook, 1981*a,b*) (Fig. 1). The concentrations of $K^+$ in normal culture medium are sufficient to approach saturation of the Na,K pump at its external $K^+$ site ($K_{1/2}$ = 0.7–1.5 mM, depending on cell type) (Sachs & Welt, 1967; Haber & Loeb, 1983; Haber et al., 1987). Reductions of the external $K^+$ to concentrations below that required to maximally activate the Na,K pump decreases the pump-mediated transport of $Na^+$ and $K^+$. The continued presence of passive leaks for these ions results in a net loss of intracellular $K^+$ and a net gain of $Na^+$ (Graves & Wheeler, 1982; Kim et al., 1984). The increase in the intracellular concentration of $Na^+$ is responsible for "short-term" regulation of the enzyme (left side of Fig. 1). With rare exceptions, the cytosolic concentration of $Na^+$ is similar to the $K_{1/2}$ for activation of the Na,K pump by intracellular $Na^+$: 10–15 mM (Garay & Garrahan, 1973; Haber & Loeb, 1983; Haber et al.,

**Key Words** Na,K-ATPase · cell culture · biosynthesis · turnover · recruitment · mRNA · intracellular signals

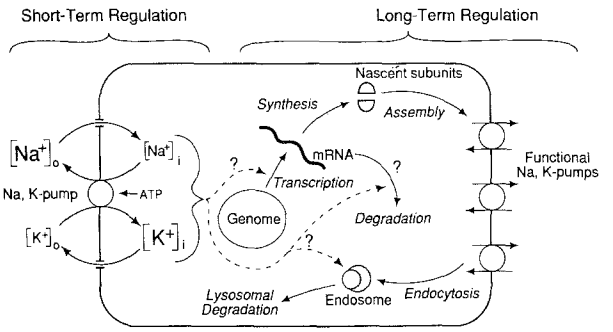


**Fig. 1.** Model for the ion concentration-dependent regulation of the Na,K pump in cultured cells. In short-term regulation (*left*), an increase in the intracellular concentration of $Na^+$ stimulates the Na,K pump via its internal $Na^+$ binding site. In long-term regulation (*right*), the abundance of the Na,K pump is increased by changes in protein synthesis and turnover. (*Adapted from* Pollack et al. (1981*b*) with permission of the publisher)

1987; Sejersted, Wasserstrom & Fozzard, 1988). The concentrations of the other substrates required by the pump (e.g., intracellular ATP and $Mg^{2+}$), in contrast, appear to be well above their respective $K_{1/2}$'s under normal conditions. As a consequence, the increase in the intracellular concentration of $Na^+$ caused by low-$K^+$ treatment is thought to activate the Na,K pump, thereby opposing the initial inhibition caused by the reduction in external $K^+$ concentration (Boardman et al., 1974; Pollack et al., 1981*a*). This short-term regulation may be sufficient to minimize dissipation of the ion gradients across the cell membrane, but at the cost of a higher-than-normal intracellular concentration of $Na^+$.

With continued exposure to low-$K^+$ medium, the deranged intracellular concentrations of $Na^+$ and $K^+$ return to near-normal values (Graves & Wheeler, 1982; Pressley et al., 1986). Short-term regulation by stimulation of the internal $Na^+$ site on the Na,K pump cannot explain this recovery of intracellular cation concentrations. Instead, the recovery is mediated by a "long-term" regulatory mechanism: an increase in the number of Na,K pumps that results in an enhanced transport capacity (right side of Fig. 1) (Pollack et al., 1981*a,b*; Kim et al., 1984; Ismail-Beigi et al., 1988). Such an up-regulation of pump abundance is also observed as a result of exposure to sublethal concentrations of digitalis glycosides or to agents that facilitate the entry of $Na^+$ (Boardman et al., 1972, 1974; Wolitzky & Fambrough, 1986). Indeed, the glycoside studies of Boardman et al. (1972) were probably the first report of up-regulation of the Na,K pump in cultured cells. In common with exposure to low external $K^+$, digitalis glycosides and agents that facilitate $Na^+$ entry elicit a partial dissipation of the original $Na^+$ and $K^+$ gradients. Because of their physiological similarities, I will refer to these treatments collectively as ion concentration-dependent stimulation. The remainder of this review will summarize the evidence for an increase in Na,K-pump number and transport capacity evoked by exposure to one of these treatments and will then describe what is known about the underlying mechanism for that increase.

## Enhanced Transport Results from Increased Na,K-Pump Abundance

The results from numerous studies indicate that an increased number of Na,K pumps provides the basis for the enhanced transport capacity produced by ion concentration-dependent stimulation. In response to low-$K^+$ medium, the number of high-affinity binding sites for the digitalis glycoside, ouabain, increases by about 60% in cultured chick heart cells and approximately doubles in HeLa cells (Pollack et al., 1981*a*; Kim et al., 1984). A doubling of another index of pump abundance, $Na^+$-dependent, $K^+$-sensitive phosphorylation, is observed in the membranes of HeLa cells in response to low-$K^+$ exposure (Pollack et al.,1981*a*), as well as in the rat liver-derived cell line (ARL 15) that my colleagues and I have investigated (Ismail-Beigi et al., 1988). Studies using polyclonal antibodies directed against the two subunits of the Na,K pump have shown an increase in antigen in Madin-Darby canine kidney (MDCK) cells after exposure to low-$K^+$ medium (Bowen & McDonough, 1987). Similar increases in antigen have been observed with pump-specific monoclonal antibodies in cultured chick skeletal muscle after exposure to veratridine, an activator of the voltage-sensitive $Na^+$ channel (Wolitzky & Fambrough, 1986).

That the increased number of Na,K-pump sites evoked by ion concentration-dependent stimulation represents functional transport proteins is supported by studies of enzymatic activity and pump-mediated transport. In crude homogenates of Chinese hamster ovary and ARL 15 cells, low-$K^+$ treatment causes a near doubling of the specific activity for Na,K-dependent adenosine triphosphatase (E.C. 3.6.1.3, Na,K-ATPase), the enzymatic equivalent of the Na,K pump (Graves & Wheeler, 1982; Pressley et al., 1986). Increases in ouabain-sensitive $Rb^+$ uptake have been observed in low-$K^+$-treated HeLa and ARL 15 cells when measured under conditions in which the Na,K pump is maximally stimulated by intracellular $Na^+$ and extracellular $K^+$—a condition thought to approach $V_{max}$ for transport (Pollack et al., 1981*a*; Pressley et al.,

**Table.** Rat Na,K-ATPase $\alpha$ isoforms

| | Mol. mass[a] (daltons) | Length[a] (amino acids) | Associated substrate affinities | | Sequence similarity to $\alpha$1 | | mRNA length (nucleotides) |
|---|---|---|---|---|---|---|---|
| | | | Ouabain (M) | $Na^+$ (mM) | Coding nucleotides (%) | Amino acids[a] (%) | |
| $\alpha$1 | 112,573 | 1018 | $10^{-5}$ | 17 | — | — | 3700 |
| $\alpha$2 | 111,736 | 1015 | $10^{-8}$ | 52 | 76 | 86 | 3400,5300 |
| $\alpha$3 | 111,727 | 1013 | ? | ? | 76 | 85 | 3700 |

Data from Lytton et al. (1985), Shull et al. (1986), and Young and Lingrel (1987).
[a] Predicted from mRNA sequence.

1986). Comparisons of Na,K-pump abundance, Na,K-ATPase specific activity, and ouabain-sensitive $Rb^+$ uptake in ARL 15 cells performed by my colleagues and I have shown that the increase in abundance occurs without a change in catalytic turnover (Ismail-Beigi et al., 1988). This suggests that the new pumps produced by ARL 15 cells in response to ion concentration-dependent stimulation are indistinguishable enzymatically from the original pumps.

We examined this issue because of the possibility that low-$K^+$ treatment produced alternative forms of the Na,K pump rather than a change in abundance. In tissues from which the Na,K pump has been purified to near-homogeneity, the enzyme complex consists of equimolar amounts of two dissimilar subunits: a 110,000-dalton $\alpha$ subunit that contains the binding sites for the substrates required for catalysis and a 40,000-60,000-dalton $\beta$ subunit that is glycosylated (Jørgensen & Skou, 1971; Hokin et al., 1973). Evidence accumulated over the last ten years indicates that the subunits of the Na,K pump exist as multiple isoforms (Table). Two isoforms of the $\alpha$ subunit that differ in electrophoretic mobility are detectable in denaturing polyacrylamide gels of Na,K-ATPase isolated from mammalian brain and adipose tissue (Sweadner, 1979; Lytton, Lin & Guidotti, 1985). Although the nomenclature is still under development, these isoforms have been tentatively designated $\alpha$1 (the form originally isolated from the kidney and described previously as the $\alpha$ subunit) and $\alpha$2 (the slower-migrating form described previously as the $\alpha(+)$ subunit). The presence and abundance of these two isoforms correlates with differences in substrate affinities and sensitivity to hormonal regulation (Lytton, 1985). In addition, a third $\alpha$ isoform ($\alpha$3) has been detected in rat brain (Shull, Greeb & Lingrel, 1986; Urayama & Sweadner, 1988). The three $\alpha$ isoforms differ in their primary structure and are encoded by different mRNAs.

The other component of the functional pump, the $\beta$ subunit, may also exist in multiple forms. Comparisons of mRNA abundance in a number of rat tissues indicates that the tissue distribution of the mRNA encoding the $\beta$ subunit is different from that of the mRNAs encoding the various $\alpha$ isoforms (Young & Lingrel, 1987; Emanuel et al., 1987). This may reflect the expression of additional isoforms of $\beta$ that are not detectable with DNA probes complementary to the known $\beta$ subunit. Further support for alternative forms of the $\beta$ subunit derives from the use of antibodies directed against the Na,K-ATPase. In chicken fibroblasts and rat liver, monoclonal antibodies fail to detect the known form of the $\beta$ subunit, despite the detection of the $\alpha$ subunit (Fambrough & Bayne, 1983; Hubert et al., 1986). Heterogeneity may also be introduced by differences in glycosylation of the $\beta$ polypeptide. The possibility of changes in the $\alpha$ and $\beta$ isoforms expressed by stimulated cells has greatly increased the potential complexity of the ion concentration-dependent response. Such a shift in isoform expression does not appear to occur in ARL 15 cells, however. The mRNA that encodes the $\alpha$1 isoform is the only species detected in these cells under both control and low-$K^+$-treated conditions (Ismail-Beigi et al., 1988).

## Synthesis and Turnover Control Abundance

Given an increase in the abundance of a membrane protein, there are at least three mechanisms that can account for that increase: (i) biosynthesis of new proteins, (ii) decreased turnover of existing proteins, or (iii) recruitment of existing proteins from some previously inactive pool, possibly intracellular. Biosynthesis might seem, at first glance, to be an unlikely mechanism for up-regulation in response to ion concentration-dependent stimulation because of the dependency of in vitro protein trans-

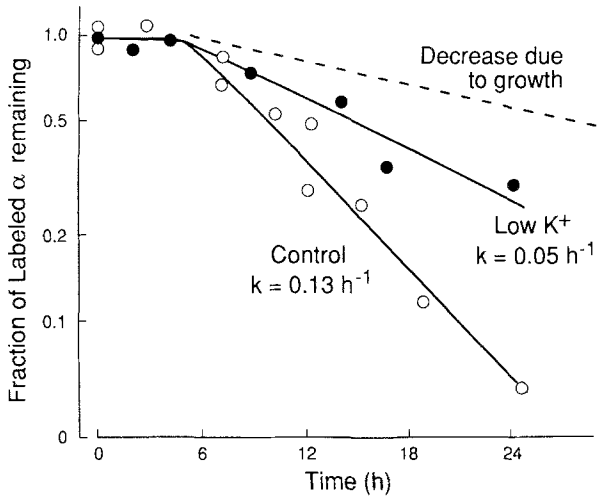


**Fig. 2.** Degradation of the $\alpha$ subunit in HeLa cells exposed for 2 days to control (○) or low (●) concentrations of external $K^+$. Cells were labeled with $^{13}C$-amino acids, then transferred to $^{12}C$-amino acids to begin the experiment. The ordinate shows the amount of labeled $\alpha$ relative to the total amount of $\alpha$ subunit. *Dashed line:* Expected decrease in label attributable to growth. (*Adapted from* Pollack et al. (1981*b*) with permission of the publisher)

lation on $K^+$ (Tse & Taylor, 1977; Weber et al., 1977). Indeed, low concentrations of external $K^+$ inhibit growth and protein synthesis in mouse fibroblasts and dog kidney cells (Pollack & Fisher, 1976; McRoberts, Tran & Saier, 1983). Furthermore, the incorporation of labeled leucine into protein is inhibited by concentrations of ouabain sufficient to block catalytic turnover of the Na,K pump (Cook et al., 1975). In contrast to the expectation, however, enhanced biosynthesis of the Na,K pump, as well as decreased turnover of the complex, have been implicated in ion concentration-dependent stimulation of Na,K transport.

In the mid 1970's, Cook and his colleagues performed a series of studies on the binding and internalization of ouabain in HeLa cells (Will et al., 1977). By labeling the pumps with bound glycoside, they measured the rate of internalization of the pump-glycoside complex, from which they inferred the turnover rate for the Na,K pump. For this inference to be valid, however, it was necessary that the binding of ouabain to the Na,K pump did not effect the subsequent probability of degradation. To test this assumption, as well as to assess the effect of low-$K^+$ treatment on protein turnover, Cook and his coworkers first incubated HeLa cells in medium supplemented with $^{13}C$-amino acids to density label the proteins (Pollack et al., 1981*b*). After returning the cells to medium containing $^{12}C$-amino acids, they monitored the amount of labeled $\alpha$ subunit remaining at various times. From these results, they estimated the half-time for the Na,K pump to be 5.4 hr—a value similar to that estimated from the internalization of bound ouabain and corresponding to a degradative rate constant of 0.13 $hr^{-1}$ (Fig. 2). Because $Na^+$-dependent, $K^+$-sensitive phosphorylation was used to localize the $\alpha$ subunit, this result probably represents the rate of turnover for the functional pump complex. In contrast to the control cells, the half-time of the pump in cells exposed to low-$K^+$ medium for 2 days was found to be 12.8 hr. Cook and his colleagues found that the decrease in the degradative rate constant to 0.05 $hr^{-1}$ in response to low-$K^+$ treatment was sufficient to account for the observed increase in Na,K-pump abundance. They concluded that a change in synthetic rate was not necessary.

There was some concern that the turnover rates estimated in Cook's laboratory were much faster than the rates of 0.005 and 0.009 $hr^{-1}$ estimated for the $\alpha$ and $\beta$ subunits, respectively, in rat renal cortex (Lo & Edelman, 1976; Lo & Lo, 1980). Subsequent experiments by Karin and Cook (1986) on the rat hepatoma line HTC using polyclonal antibodies directed against the Na,K-ATPase found a degradative rate constant for the $\alpha$ subunit of 0.015 $hr^{-1}$—a rate of turnover that is less than HeLa cells but greater than kidney cortex. In studies of chick sensory neurons in culture, Tamkun and Fambrough (1986) estimated that the degradative rate constant for the pump complex was 0.017 $hr^{-1}$. It therefore seems probable that the turnover of the Na,K pump may vary by 30-fold or more in different cells.

A limitation of the techniques used to measure turnover is the long period of time (relative to the time course of up-regulation) necessary to monitor the decline in label. A transient increase in the synthetic rate might go undetected if it occurred early in the response. Wolitzky and Fambrough (1986) took advantage of monoclonal antibodies directed against the $\beta$ subunit of the Na,K-ATPase of chicken to monitor protein synthesis. They measured the synthetic rate of the pump complex in cultured chick skeletal muscle cells during treatment with an activator of voltage-sensitive $Na^+$ channels, veratridine (Fig. 3). Incorporation of [$^{35}S$]methionine into the $\alpha$ and $\beta$ subunits nearly doubled after 12–18 hr of treatment, but the increase was transient and returned to control levels by 36 hr. In contrast, the 60% increase in immunodetectable Na,K pumps required 30 hr to reach a maximum. Because the abundance of detectable pumps was still increasing while the synthetic rate was declining from its initial stimulation, Wolitzky and Fambrough reasoned that a decrease in the turnover rate must also accompany the response to

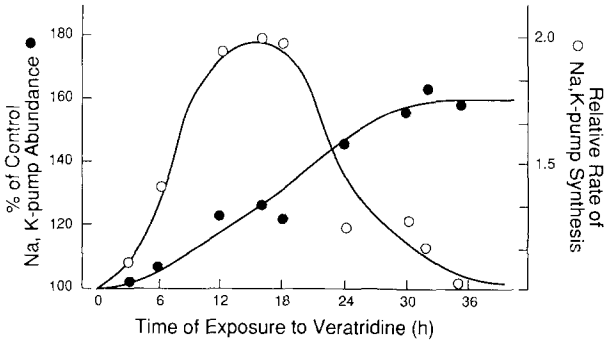


**Fig. 3.** Up-regulation and biosynthesis of the Na,K pump in cultured chick muscle cells exposed to 10 mM veratridine. Cells were pulse labeled with [$^{35}$S]methionine at each of the time points. Abundance (●) and synthesis (○) of the Na,K pump are relative to control cells. (*Redrawn from* Wolitzky & Fambrough (1986) with permission of the publisher)

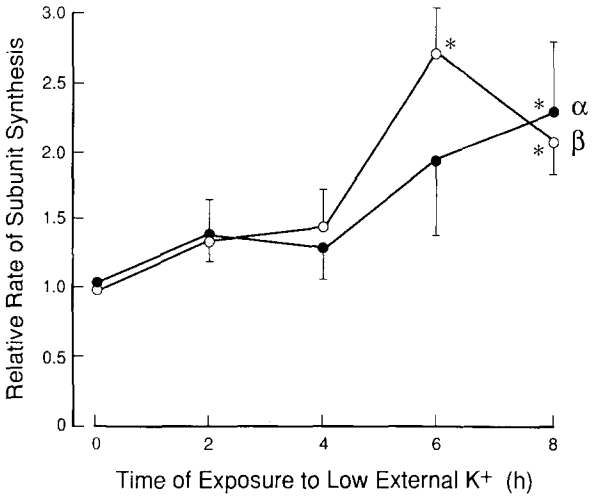


**Fig. 4.** Biosynthesis of the constituent subunits of the Na,K pump in MDCK cells exposed to low concentrations of external $K^+$. Cells were pulse labeled with [$^{35}$S]methionine at each of the time points. Synthetic rates of the $\alpha$ (●) and $\beta$ (○) subunits are relative to control cells. (*Redrawn from* Bowen & McDonough (1987) with permission of the publisher)

veratridine. Subsequent experiments confirmed that the degradative rate constant for the pump complex declined from 0.022 $hr^{-1}$ in control cells to 0.011 $hr^{-1}$ in cells treated for 12 hr with the drug. They concluded that up-regulation of the Na,K pump in response to veratridine stimulation involved a transient increase in the synthetic rate followed by a decrease in the degradative rate.

The work of Wolitzky and Fambrough shows that ion concentration-dependent stimulation of Na,K-pump abundance is mediated, at least in part, by an increase in the synthetic rate for the pump complex. It is not clear, however, what effects stimulation has on the synthetic rates of the individual subunits. Immunoprecipitation of the $\alpha$ subunit with the monoclonal antibody used by Wolitzky and Fambrough depends on the association of each $\alpha$ with a $\beta$ subunit, the antigen recognized by the immunoglobulin. The synthetic rate for the $\alpha\beta$ complex, however, may be a complicated function of the synthetic rates for the individual subunits and the rate of their association (although Wolitzky and Fambrough concluded that the latter must be very fast). More recent experiments performed by Bowen and McDonough (1987) address this problem. They used polyclonal antibodies directed against the holoenzyme to measure the synthetic rates of the individual subunits in MDCK cells challenged with low external $K^+$ (Fig. 4). After 8 hr in low-$K^+$ medium, the synthetic rates of the $\alpha$ and $\beta$ subunits were more than doubled. Because the antibodies recognize both subunits, the measured rates are probably not dependent on the association of $\alpha$ with $\beta$.

Of the three potential mechanisms for an increase in pump abundance, recruitment from an inactive pool remains to be discussed. These recruitment processes can be divided into two groups: (i) mechanisms that involve conversion of a nonfunctioning pump or its constituents into an active pump by allosteric or covalent means, and (ii) mechanisms that involve transfer of potentially functional pumps from a protected or "masked" site to the plasma membrane. These protected sites are usually presumed to be membrane bound and intracellular. Evidence for protected sites might be demonstrated by an increase in the apparent activity of the Na,K-ATPase in cell lysates following treatment with detergents, which presumably open impermeable vesicles (Jørgensen & Skou, 1971). Studies by Pollack et al. (1981*a*) on HeLa cells, as well as our own studies on ARL 15 cells (Ismail-Beigi et al., 1988), have shown that detergent treatment of membranes increases Na,K-ATPase activity by 50–70% (Fig. 5). This increase cannot explain the up-regulation of Na,K-pump abundance elicited by low-$K^+$ treatment, however, because detergent-mediated activation is similar in the membranes of control and low-$K^+$-treated cells. The use of immunological reagents provides additional evidence against recruitment from an intracellular pool as the basis of up-regulation. Wolitzky and Fambrough (1986) used monoclonal antibodies to show that intracellular sites account for about 60% of the detectable $\beta$ subunit in cultured chick skeletal muscle cells. In response to veratridine, both cell surface and intracellular pools of immunologically detectable material increased, indicating that the increase in pump abundance was not caused by transfer to the plasma

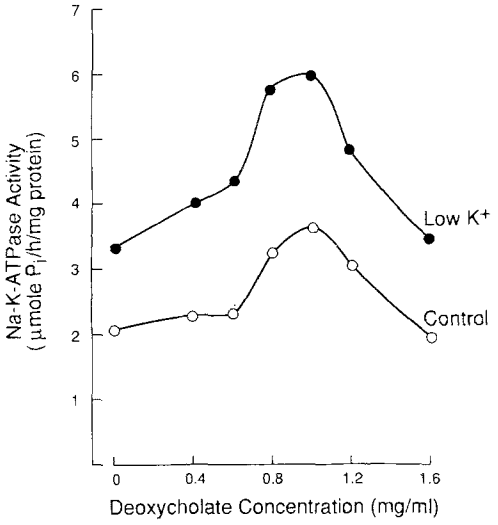


**Fig. 5.** Deoxycholate activation of Na,K-ATPase activity in ARL 15 cells exposed for 24 hr to control (○) or low (●) concentrations of external $K^+$. Crude membrane fractions were isolated and preincubated with detergent prior to enzymatic assay. (*Redrawn from* Ismail-Beigi et al. (1988) with permission of the publisher)

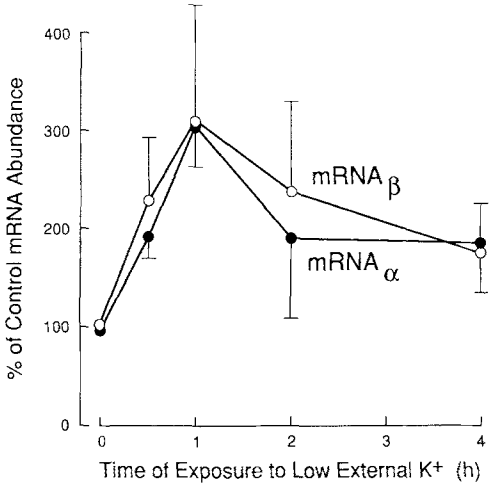


**Fig. 6.** Abundance of the mRNAs encoding the constituent subunits of the Na,K pump in MDCK cells exposed to low concentrations of external $K^+$. DNA probes complementary to $mRNA_\alpha$ (●) and $mRNA_\beta$ (○) were hybridized to total RNA in dot blots. The ordinate shows the abundance relative to control cells. (*Redrawn from* Bowen & McDonough (1987) with permission of the publisher)

membrane from an intracellular site. The increase in the number of antigenic sites on the plasma membrane also implies that the elevated number of Na,K pumps is not caused by the conversion of inactive to functional pumps. If the inactive Na,K pumps do not differ significantly from functional pumps at the epitope recognized by the monoclonal antibodies, such inactive pumps should be detectable. Together with the detergent-activation experiments, the results from these immunological experiments suggest that recruitment from an inactive pool does not account for up-regulation of the Na,K pump.

## Increased Abundance of $mRNA_\alpha$ and $mRNA_\beta$ May Stimulate Synthesis

An enhanced synthetic rate such as that observed for the $\alpha$ and $\beta$ subunits of the Na,K pump might be caused by an increase in the abundance of the mRNAs that encode the subunits or an increase in the efficiency of their translation. The isolation of complementary DNA probes specific for $mRNA_\alpha$ and $mRNA_\beta$ (the mRNAs that encode the $\alpha$ and $\beta$ subunits, respectively) has provided the tools necessary to evaluate these possibilities. Using cDNA probes derived from dog kidney, Bowen and McDonough (1987) measured the relative abundance of $mRNA_\alpha$ and $mRNA_\beta$ in low-$K^+$-treated MDCK cells. Within 1 hr after reduction of the external $K^+$, they found that the abundance of both $mRNA_\alpha$ and $mRNA_\beta$ increased by about threefold in samples of total RNA (Fig. 6). At later time periods, however, the abundance of the two mRNA species declined, so that by 4 hr they were twice their initial values. This transient threefold increase in abundance of the mRNAs preceded the increase in synthetic rates for the subunits—as might be expected if the increase in mRNA abundance was the basis for the increase in synthetic rate. Moreover, the changes in mRNA abundance appeared to be coordinate in MDCK cells.

Our results in ARL 15 cells further support an increase in mRNA abundance in response to reduced concentrations of external $K^+$ (Pressley et al., 1988). We employed cDNAs derived from rat brain (Schneider et al., 1985; Mercer et al., 1986) as hybridization probes in blots of total and poly($A^+$)RNA. Northern blots of total RNA isolated from cells exposed to control or low-$K^+$ medium revealed a single band with a migration rate appropriate for $mRNA_\alpha$ (26–27 S) (Fig. 7). Subsequent studies have shown that this mRNA encodes the $\alpha 1$ isoform (Ismail-Beigi et al., 1988). Exposure of cells to low-$K^+$ medium for 6 hr elicited 60% increases in the abundance of $mRNA_\alpha$ and $mRNA_\beta$ relative to cells exposed to control medium. By 24 hr, the abundance of $mRNA_\alpha$ had increased by 290%, but $mRNA_\beta$ had increased by only 70%, indicating that in ARL 15 cells, regulation of Na,K-pump mRNAs is not coordinate. Moreover, the abundance of $mRNA_\beta$ was 30-fold less than that of $mRNA_\alpha$ in

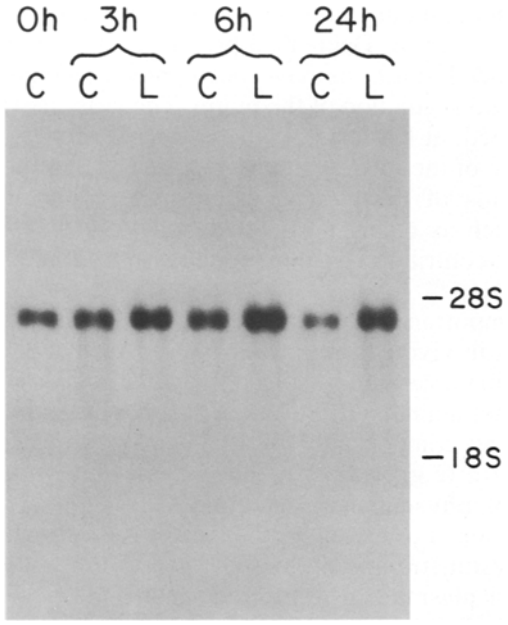


**Fig. 7.** Abundance of the mRNA encoding the $\alpha$ subunit of the Na,K pump in ARL 15 cells exposed to control (*C*) or low (*L*) concentrations of external $K^+$. DNA complementary to $mRNA_\alpha$ was hybridized to a Northern blot of total RNA. Size markers represent the positions of the 28 S and 18 S ribosomal subunits. (*From* Pressley et al. (1988) with permission of the publisher)

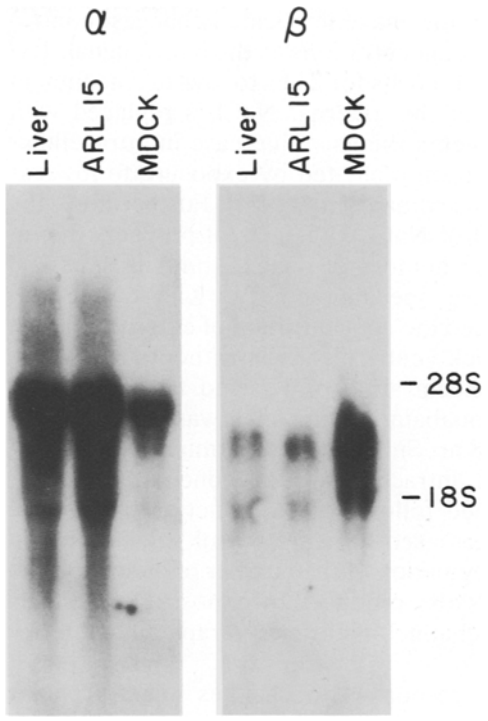


**Fig. 8.** Comparison of the mRNAs encoding the constituent subunits of the Na,K pump in rat liver, ARL 15 cells, and MDCK cells. DNAs complementary to $mRNA_\alpha$ ($\alpha$) and $mRNA_\beta$ ($\beta$) were hybridized to Northern blots of total RNA. Size markers represent the positions of the 28 S and 18 S ribosomal subunits. (*From* Pressley et al. (1988) with permission of the publisher)

ARL 15 cells treated with control medium. Similar discrepancies between the abundance of $mRNA_\alpha$ and $mRNA_\beta$ were found in total RNA isolated from rat liver, but not from MDCK cells, suggesting that the difference in abundance may be specific to liver and liver-derived cell lines (Fig. 8). These differences in abundance were surprising because the near-homogeneous samples of Na,K-ATPase purified from high-activity tissues (e.g., mammalian renal outer medulla, shark rectal gland) consist of equimolar amounts of the two constituent subunits (Jørgensen & Skou, 1971; Hokin et al., 1973). If this is also true in ARL 15 cells, then the difference in mRNA abundance suggests that additional regulatory mechanisms such as changes in translational efficiency may contribute to ion concentration-dependent stimulation. Alternatively, liver and liver-derived cell lines may express an additional form of $\beta$ subunit that is not detected by the cDNA probe. The existence of additional forms of the $\beta$ subunit is also suggested by the absence of immunodetectable $\beta$ in fibroblasts and liver (Fambrough & Bayne, 1983; Hubert et al., 1986).

The increase in the abundance of the mRNAs encoding the subunits of the Na,K pump might be the result of an enhanced rate of nuclear transcription and processing or a decrease in the rate of degradation. Conceptually, the problem is analogous to that posed by the increase in pump number discussed earlier. Currently, several laboratories are assessing the role of transcription in ion concentration-dependent regulation. The most widely used technique for estimating transcription rates, nuclear run-on, measures the incorporation of radiolabeled ribonucleotides into nascent hnRNA chains that were initiated in the nucleus prior to its isolation (McKnight & Palmiter, 1979). Preliminary results from McDonough et al. (1988) show that exposure of MDCK cells to low external $K^+$ elicits an increase in the nuclear run-on of the $mRNA_\alpha$ precursor, suggesting that increased nuclear transcription may account for at least part of the increase in mRNA. As of yet, however, a complete description of ion concentration-dependent effects on RNA transcription has not appeared.

## $Na^+$ May Be the Intracellular Signal

Until now I have discussed the mechanisms by which the number of Na,K pumps are regulated in response to changes in ion concentrations, but it remains to discuss the initial signal that initiates the

response. Studies have implicated changes in intracellular $Na^+$ concentrations as the initial signal. Exposure of HeLa cells for 24 hr to low-$K^+$ medium in which 40% of the external NaCl is replaced with sorbitol prevents the usual increase in intracellular $Na^+$ concentration elicited by exposure to low external $K^+$ (Boardman et al., 1974). Furthermore, the replacement of NaCl with sorbitol prevents the increase in the number of ouabain-binding sites and Na,K-ATPase specific activity. Kim and Smith (1986) reduced the concentration of external $Na^+$ on cultured chick ventricular cells without altering the external concentration of $K^+$ and found that the number of ouabain-binding sites was decreased after 24 and 48 hr. Subsequent determinations demonstrated that intracellular $Na^+$ concentrations initially declined following the reduction in external $Na^+$, but increased to near control values after 48 hr. Down-regulation of the number of ouabain-binding sites was also produced by exposure of the cells to the $Na^+$ channel inhibitors, verapamil and tetrodotoxin.

Cellular responses to changes in intracellular $Na^+$ concentration are difficult to interpret because of the number of additional factors that change as well. A short list of these factors includes intracellular pH, intracellular $Ca^{2+}$ concentration, cell volume, and the transmembrane electrical potential. Rather than continue to search directly for the signal that initiates the ion concentration-dependent response, a complementary approach may be provided by the recent isolation of the genes that encode the subunits of the Na,K pump (Shull & Lingrel, 1987). Changes in nuclear transcription, if shown to mediate the up-regulation of the mRNAs that encode the Na,K pump, must ultimately reflect changes in the binding of regulatory proteins to the genes. Based on the nucleotide sequences of the genes encoding the subunits of the pump, potential regulatory sequences might be identified based on similarities with known regulatory regions. Should changes in mRNA degradation prove to be the basis for the increase in mRNA abundance, then the appropriate regulatory sequences must reside on the mature mRNA molecule itself. Identification and analysis of the binding proteins responsible for regulation in model systems may allow us to work towards the signal that initiates the response, rather than choosing from a long list of possible signals.

## Summary and Concluding Remarks

Up-regulation of the number of Na,K pumps elicited by changes in the concentrations of extra- and intracellular $Na^+$ and $K^+$ requires the regulation of multiple subunits at the levels of both protein and mRNA abundance. Decreased protein degradation and enhanced synthesis have been shown to contribute to up-regulation of the pump. The latter may be explained, at least in part, by an increase in the abundance of the mRNAs that encode the constituent subunits of the enzyme complex. Additional factors such as changes in translational efficiency may also contribute to the increase in synthetic rate.

The importance of ion concentration-dependent regulation in vivo is not known, but the regulatory mechanisms observed in culture may also function in the intact animal. Although the response may be modified by compensatory mechanisms not available in vitro (e.g., endocrine and neural inputs), a number of physiological conditions might initiate up-regulation. For example, the loss of $K^+$ through kidney dysfunction or the chronic use of diuretics may lower plasma and intracellular concentrations of $K^+$ and elicit an ion concentration-dependent response in a wide variety of tissues. Similarly, the administration of digitalis glycosides during congestive heart failure probably results in an increased Na,K-pump abundance. Given the possibility that these homeostatic mechanisms may operate in response to pathology, the study of ion concentration-dependent regulation of the Na,K pump may yield insights into the effects of disease on this important transport system.

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