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Serum Independence of Low K^+ Induction of Na,K-ATPase: Possible Role of *c-fos*

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Summary. Cultured ARL_{15} cells respond to abnormally low extracellular K^+ concentrations by increasing the abundance of Na,K-ATPase (the Na/K pump). This response is preceded by significant increases in the mRNAs of the $\alpha 1$ and $\beta 1$ subunits of this enzyme, implying transcriptional or post-transcriptional regulation in the response. The present study concerned the possible participation of serum factors in low K^+ induction of Na,K-ATPase.

In normal K^+ (4.5 mM) or low K^+ (0.68 mM) the presence of 10% calf serum had no effect on Na,K-ATPase activity. The serum independence of the response to low K^+ raised the possibility that low K^+ may itself elicit a "growth" response. Accordingly, the effect of low K^+ on mRNA abundances of four proto-oncogenes (*c-fos*, *c-myc*, *c-jun* and *c-ski*) was evaluated in the early phase of the response by quantitative Northern blot analysis. The mRNA for *c-fos* was transiently elevated by low K^+, with a peak at 30 min. In contrast, low K^+ had no measurable effect on the abundances of *c-myc*, *c-jun* and *c-ski*, for up to 2 hr of exposure.

The early elevation of *c-fos* mRNA makes it a candidate mediator in this signal-transduction pathway. Induction of *c-fos* mRNA by the phorbol ester, PMA, or by dioctanoyl glycerol, however, had no effect on Na,K-ATPase activity. These results indicate that an increase in *c-fos* mRNA alone is not sufficient to induce Na,K-ATPase. Whether induction of *c-fos* is necessary for the response to low K^+ remains to be determined in future studies.

Key Words serum · low K^+ · Na,K-ATPase · Na/K pump · proto-oncogenes · *c-fos* · *c-myc* · *c-jun* · *c-ski*

Introduction

The Na,K-ATPase (E.C. 3.6.13) or Na/K pump is an integral membrane oligomeric protein responsible for maintaining ionic composition and cell volume (Skou, 1988). Incubation of a variety of cells in tissue culture in low concentrations of K^+ (<1 mM) results in a net loss of K^+ and a net gain of Na^+ (Graves & Wheeler, 1982; Kim et al., 1984). These changes are subsequently reversed within 12 to 24 hr by significant increases both in the abundance of Na,K-ATPase and in active transmembrane transport of Na^+ and K^+ in cells in culture (Pollack, Tate & Cook, 1981*a*,*b*; Pressley et al., 1986, 1988; Bowen & McDonough, 1987; Ismail-Beigi et al., 1988). Since there are concomitant overall increases in DNA, RNA and protein contents of the cells, the low K^+ effect is pleiotropic (Pressley et al., 1986). The response may be mediated by transcriptional events since increased abundances of mRNAs encoding the α and β subunits of the Na,K-ATPase in the presence of low K^+ occur at 6 hr, prior to the increased enzyme abundance at 12–24 hr (Pressley et al., 1988).

Thyroid hormone, T_3, also augments Na,K-ATPase activity, transcription of Na,K-ATPase α and β subunit genes, and the abundance of $mRNA_\alpha$ and $mRNA_\beta$ (Lo et al., 1976; Guernsey & Edelman, 1983; Chaudhury et al., 1987; Gick, Ismail-Beigi & Edelman, 1988). With the identification of the T_3 receptor as the gene product of the proto-oncogene, *c-erb*A, the possibility is raised that the action of both low K^+ and T_3 may be mediated via induction of a cascade of nuclear proto-oncogenes (Koenig et al., 1988; Murray et al., 1988).

In this paper, we describe the effect of serum (as a source of growth factors) on the low K^+ response in ARL_{15} cells in culture. As serum and serum growth factors induce *c-fos* and *c-myc* (Curran, Bravo & Miller, 1985; Grinstein & Rothstein, 1986; Moolenar, 1986), the effect of low K^+ on the abundance of these nuclear proto-oncogenes, as well as others, was studied. Our results show that serum does not enhance Na,K-ATPase activity in normal K^+ and that low K^+ induction of Na,K-ATPase is serum independent in this cell line. Low K^+ also independently induces *c-fos* mRNA transiently.

Materials and Methods

Materials

Culture medium and calf serum were purchased from GIBCO (Grand Island, NY). Plastic culture plates were from Corning Glassworks (Medefield, MA), guanidinium thiocyanate (GTC) from Fluka (Ronkonkoma, NY), the nick translation kit and additional recombinant DNA reagents from BRL (Gaithersburg, MD) and restriction endonucleases from Boehringer Mannheim (Indianapolis, IN). Thymidine-5′-α-[^{32}P]-triphosphate was provided by Amersham (Arlington Heights, IL). Nitrocellulose was obtained from Schleicher and Schuell (Keene, NH). Bovine serum albumin, calf thymus DNA, β-mercaptoethanol, L-ascorbic acid and EGTA were purchased from Sigma (St. Louis, MO). Ammonium molybdate, KCl and other conventional reagents were purchased from Fisher Scientific (Pittsburgh, PA).

Cell Culture

An established adult rat liver cell line, ARL_{15}, was a generous gift from the Naylor Dana Institute for Disease Prevention (Valhalla, NY) (San et al., 1979). We isolated cells between passages 25–27 that survived at confluency for up to one week in the absence of serum. These were grown to confluency in Williams Medium E (WME) supplemented with 10% calf serum in 100-mm Corning culture plates at 37°C in a 5% CO_2 atmosphere. Further experimental details are provided in the table legends.

Preparation of Crude Homogenates

The monolayers of cells were washed twice with 10 ml ice-cold homogenization buffer (HB), consisting of 50 mM Tris-Cl, pH 7.5, 1.25 mM EGTA, 250 mM sucrose. The monolayers of the individual plates were scraped into 2 ml of HB with a rubber policeman, transferred to Corning centrifuge tubes and centrifuged for 6 min at 2,000 rpm. All subsequent steps were performed at 4°C. The cells were suspended in 0.5 ml of HB, homogenized with 10 strokes of a Teflon pestle in a Potter-Elvehjem homogenizer and frozen in liquid nitrogen. Prior to freezing, a well-mixed aliquot was removed for protein measurement by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Na,K-ATPase Assay

The Na,K-ATPase assays were performed by a modification of the method of Pressley et al. (1986). The incubation solution contained (in mM): 130 NaCl, 20 KCl, 12 $MgCl_2$, 6 ATP, 25 histidine, 0.2 EGTA, 0.3 NaN_3, pH 7.5, in a total volume of 200 μl, in the absence or presence of 3 mM ouabain. Reactions were started by addition of 20 μg homogenates and proceeded for 15–30 min at 37°C. The reaction was stopped by addition of 100 μl of an ice-cold solution of 20% trichloroacetic acid and left on ice for 5 min before centrifuging in an Eppendorf microcentrifuge for 5 min. The amount of inorganic phosphate released was measured by the colorimetric method of Baginski, Foa and Zak (1967) in 250 μl of the supernatant.

Northern Blot Analysis

The abundances of specific mRNAs were analyzed by established methods (Chirgwin et al., 1979; Pressley et al., 1986). After washing the plates twice with 8 ml of ice-cold HB, RNA was isolated by denaturation with guanidine thiocyanate buffer, precipitation with LiCl, extraction in phenol/chloroform, and ethanol precipitation. The RNA was dissolved in water and stored until analysis at −20°C (Pressley et al., 1988). The mRNA was analyzed with cDNA probes by Northern blotting (Thomas, 1980). The concentration of RNA was determined spectrophotometrically at 260 and 280 nm (Maniatis, 1982). Total RNA (10–20 μg) was fractionated by electrophoresis in a 1% agarose gel containing 6% formaldehyde and irradiated with UV light for 10 min before transferring to nitrocellulose paper by capillary action (Maniatis, Fritsch & Sambrook, 1982). The blots were prehybridized and subsequently hybridized to specific radiolabeled cDNA probes for 3–4 days as described by Chaudhury et al. (1987). The specific cDNA probes were purified by digestion with appropriate restriction endonucleases [*v-fos* (SmaI); *c-myc* (XhoI); *c-jun* (HindIII and BamHI) and *v-ski* (EcoRI)], electrophoresis in 1% low melting point agarose gels and electroelution (Maniatis, 1982). The cDNA fragments were radiolabeled with thymidine 5′-α-[^{32}P] triphosphate to a specific activity of 2–6 × 10^8 cpm/μg by nick translation. After hybridization, the blots were washed for 1 hr at 55°C with four changes of 0.1*x* SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 0.1% SDS; the nitrocellulose was blotted dry and autoradiographed at −70°C using XAR-5 film (Eastman Kodak, Rochester, NY) and two Cronex Lightning Plus intensifying screens (DuPont, Wilmington, DE). The autoradiograms were developed, and the relative intensities of the bands were measured by densitometry with a Helena Quick Scan Jr. equipped with a peak area integrator (Helena Laboratories, Beaumont, TX).

Statistical Analysis

All data are expressed as means ± SEM. Statistical differences between groups were evaluated by the Student's *t* test and considered significant when $P < 0.05$.

Results

Na,K-ATPase activity (V_{max}) of confluent ARL_{15} cells (crude homogenates) was the same when the cells were incubated for 22–24 hr in WME alone, or WME containing either 10% calf serum, 1% bovine serum albumin, 1% ovalbumin or 1% human serum albumin (Table 1). These results indicate that serum growth factors and serum albumins had no effect on Na,K-ATPase activity in this cell population for up to 24 hr.

Incubation of confluent ARL_{15} cells in WME and low K^+ (0.68 mM) for 24 hr, increased Na,K-ATPase activity by 57%, when compared to that obtained in 4 mM K^+ (Table 2). Similar increases in Na,K-ATPase activity in response to low K^+ were obtained in serum-free medium or in the presence of 5% calf serum (Table 2). The concentration of K^+

in 5% serum was determined by flame photometry to range from 0.36–0.41 mM. Hence the contribution of K^+ in the serum was used to determine the final concentration of K^+ in all experiments. To verify the serum independence of the response, ARL_{15} cells were grown to confluency (5–6 days) without changing the medium during the growth period. Half of the confluent plates were then incubated for 24 hr in low K^+ or normal K^+ medium and then assayed. The remainder were incubated for 24 hr in WME (serum free) containing 4 mM K^+ and then switched to WME (serum free) containing normal or low K^+ for an additional 24 hr (Table 3). The increase in Na,K-ATPase activity in response to low K^+ after 48 hr in serum-free medium (24 + 24) was equivalent to that obtained in the presence of serum (*cf.* Tables 2 and 3).

Table 1. Effect of serum and serum albumins on Na,K-ATPase activities

	μmol P_i/hr/mg protein	
	Expt. 1.	Expt. 2.
Williams E	2.5 ± 0.1	1.4 ± 0.1
Williams E + 10% calf serum	2.7 ± 0.2	1.8 ± 0.2
Williams E + 1% bovine serum albumin	2.5 ± 0.2	1.8 ± 0.1*
Williams E + 1% ovalbumin	2.4 ± 0.2	1.4 ± 0.1
Williams E + 1% human serum albumin	2.3 ± 0.2	1.6 ± 0.2

ARL_{15} cells (passage 25) were grown to confluency in WME supplemented with 10% calf serum. The medium was changed 24 hr before confluency. When the cells reached confluency, the plates were washed with 10 ml WME and replaced with 8 ml WME for 3 hr. The medium was then replaced with either WME or WME supplemented with either 10% calf serum or 1% albumin as indicated. Crude homogenates were assayed for Na,K-ATPase activity, after 22–24 hr in the respective media. Expt. 1 was done in the absence of detergent. In expt. 2, the homogenates were incubated in the presence of 0.8 mg/ml Na deoxycholate (final concentration) for 30 min at room temperature before enzyme assay. Values are reported as means ± SEM (n = 4 plates each). Within each experiment, the values did not differ significantly from Williams E alone (i.e., $P > 0.05$) except for the value marked with an asterisk (i.e., $P < 0.025$).

The potential role of early gene expression in the response to low K^+ was studied by analyzing the message abundances of the nuclear proto-oncogenes, *c-fos*, *c-jun*, *c-myc* and *c-ski*. Thirty min after exposure to low K^+ the abundance of *c-fos* mRNA was 3.9-fold higher than cells incubated in normal K^+ (Table 4, Fig. 1). The increase in *c-fos* mRNA was transient and detected only at 30 min. No *c-fos* mRNA was detected at 0, 60 or 120 min (Fig. 1). The equivalent values for *c-myc* were 1.3, 0.9 and 1.1 (Table 4, Fig. 2). The *c-jun* mRNA low K^+/control ratios were 1.4, 1.2 and 0.9, at 30, 60 and 120 min, respectively, and those for *c-ski* were 0.9, 1.0 and 1.7. Only the relative increase (low K^+ *versus* control) of *c-fos* mRNA at 30 min was statistically significant ($P < 0.01$).

An alternative inducer of *c-fos*, PMA (phorbol-1,2-myristate-13 acetate) was used to evaluate the possibility that transient induction of *c-fos* mRNA by an alternative inducer is sufficient to evoke an increase in Na,K-ATPase activity. *c-fos* mRNA was induced 4.6-, 6.8- and 4.9-fold at 15, 30 and 60 min after exposure to 94 ng/ml PMA. As with low K^+, the induction of *c-fos* mRNA was transient with

Table 2. Effect of serum on low K^+ response

	Control	Low K	% change	P
Williams E medium				
(−DOC)	1.50 ± 0.10(4)	2.22 ± 0.08 (4)	48	<0.01
(+DOC)	1.40 + 0.07(4)	2.29 + 0.18 (4)	64	<0.01
Williams E medium + 5% serum				
(−DOC)	1.38 ± 0.10(4)	2.14 ± 0.145(4)	55	<0.01
(+DOC)	1.21 + 0.06(4)	1.98 + 0.105(4)	64	<0.01
Williams E medium + 1% BSA				
(−DOC)	1.31 ± 0.07(3)	1.8 ± 0.12 (3)	39	>0.10

ARL_{15} cells (passage 27) were grown to confluency in WME supplemented with 10% calf serum. The medium was changed 24 hr before confluency. The plates were washed twice with 10 ml Williams "K^+-free" medium. Finally 10 ml of WME containing 5% calf serum or 1% BSA or no additions and K^+ concentrations of either 0.68 mM (low K^+) or 4 mM (control) were added to the plates for 24 hr. In the assays done after treatment of the homogenates with detergent, the concentration of Na deoxycholate (DOC) was 0.8 mg/ml and was preincubated for 30 min at room temperature. Na,K-ATPase specific activity is expressed as μmol P_i released per hr per mg protein. Values are reported as means ± SEM. The number of plates assayed is given in parentheses.

Table 3. Effect of low K^+ on Na,K-ATPase activity in the absence of serum

Time in serum-free medium (hr)	Control	Low K treatment	% change	*P*
24	1.5 ± 0.04	1.9 ± 0.1	27	<0.01
24 + 24	1.4 ± 0.1	2.1 ± 0.1	51	<0.01

ARL_{15} cells (passage 26) were plated at a concentration of 10% in WME containing 10% calf serum and grown to confluence (5–6 days) without any change in medium. The plates were washed twice with 10 ml Williams "K^+-free" medium. The plates were divided into two groups. The first group received WME containing 4 mM K^+ (control) or 0.68 mM (low K^+) and incubated for 24 hr before assay for Na,K-ATPase activity. WME containing 4 mM K^+ was added to the second group of plates, and these were left for an additional 24 hr (serum free) before washing them again with 10 ml WME K^+-free medium and subsequently adding 10 ml of WME containing 4 mM K^+ (control) or 0.68 mM K^+ (low K^+) and incubating for another 24 hr before assaying for Na,K-ATPase activity (expressed as μmol P_i/hr/mg protein). These latter samples are referred to as (24 + 24 hr). Values are reported as means ± SEM. n = 4 plates in each group.

Table 4. Effect of low K^+ on the relative abundance of mRNAs encoding nuclear proto-oncogenes

	Relative increase (low K^+/control)		
	30 min	60 min	120 min
c-fos	3.90 ± 0.88(11)	0(11)	0(11)
c-jun	1.43 ± 0.21(8)	1.23 ± 0.18(8)	0.93 ± 0.18(5)
c-myc	1.27 ± 0.17(8)	0.94 ± 0.16(9)	1.12 ± 0.26(5)
c-ski	0.94 ± 0.20(8)	0.99 ± 0.24(9)	1.70 ± 0.40(6)

ARL_{15} cells (passages 25–27) were plated at 10% confluency in WME containing 10% calf serum and allowed to reach confluency (5–6 days) without any change in medium. The plates were washed twice with 10 ml of WME K^+-free medium and then incubated in 8 ml WME K^+-free medium supplemented to 4 mM K^+ for an additional 24 hr. The cells were again washed twice with 10 ml Williams K^+-free medium and subsequently received 8–10 ml WME containing K^+ concentrations of 4 mM (control) or 0.68 mM (low K^+) for 30, 60 and 120 min before processing for mRNA isolation as described by Pressley et al. (1986). Total RNA extracts were resolved by electrophoresis and probed with radiolabeled cDNA probes. The Northern blot analyses were quantified by densitometry, and all results are expressed as the ratio of low K^+ value/control at each time point. All values are mean ± SEM. The number of plates assayed is given in parentheses.

maximum abundance at 30 min and a return to an undetectable concentration at 120 min (Fig. 3). This effect, however, was more pronounced with PMA than with low K^+. Transient but much smaller increases in *c-fos* mRNA were seen in the controls as well. PMA had no effect on *c-myc* concentrations for up to 2 hr (*data not shown*). Treatment with

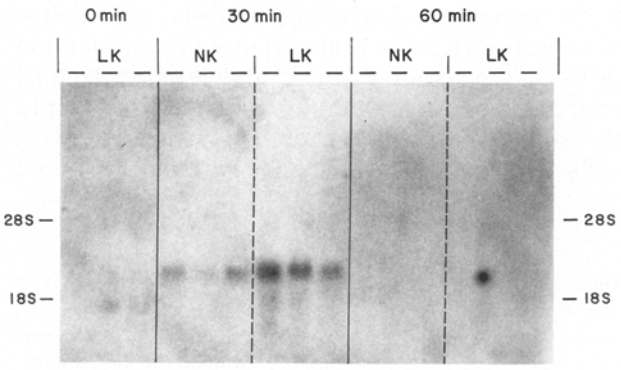

Fig. 1. A representative Northern blott of *c-fos* mRNA from normal or low K^+ treated cells. ARL_{15} cells (passage 25) were plated, treated and exposed to normal 4 mM (NK) or 0.68 mM (LK) external K^+ for various lengths of time as described in the legend of Table 4. Size markers represent the positions of the 28S and 18S ribosomal subunits.

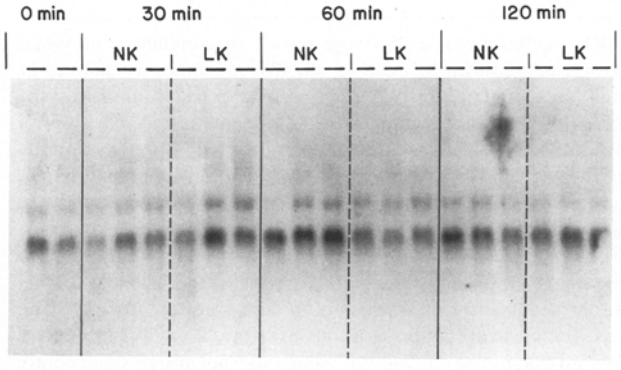

Fig. 2. A representative Northern blot of *c-myc* mRNA isolated from normal or low K^+ treated ARL_{15} cells. The experimental details are described in the legends of Fig. 1 and Table 4.

PMA (94 ng/ml) or dioctanoyl glycerol (200 μM), an alternative inducer of protein kinase C, for 24 hr had no effect on Na,K-ATPase activity of confluent monolayers of ARL_{15} cells (Table 5).

Discussion

Cell lines in tissue culture commonly require the presence of 5 to 10% serum for growth and replication. The presence of an abundance of growth factors in serum, some of which have been identified (e.g., EGF, FGF, PGDF, insulin, triiodothyronine), presumably accounts for this requirement (Gospodarowicz & Moran, 1974). The observation that cell growth and division was accompanied by altered selective ion permeabilities in ARL_{15} cells, raised

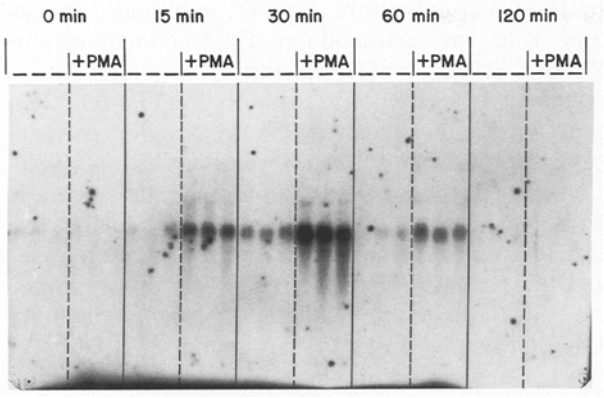

Fig. 3. A representative Northern blot of *c-fos* mRNA induction at 15, 30, 60 and 120 min in ARL_{15} cells by PMA. Note that the change in medium alone enhanced the yield to above background. ARL_{15} cells were plated at 10% confluence in WME containing 10% calf serum and allowed to attain confluency without any change of medium. PMA (93.7 ng/ml) in 0.0093% DMSO or DMSO alone were then added to the plates, and the cells were harvested at the indicated times. The experimental details are described in the legends of Fig. 1 and Table 5.

Table 5. Effect of PMA (phorbol-1,2-myristate-13-acetate) and dioctanoyl-glycerol on Na,K-ATPase activity

	Specific activity (μmol P_i/hr/mg (protein)
a. WME	2.18 ± 0.14
DMSO (0.0093%)	2.28 ± 0.16
PMA (93.7 ng/ml)	2.03 ± 0.07
b. WME	2.90 ± 0.12
Dioctanoyl glycerol (200 μM)	2.80 ± 0.09

ARL_{15} cells (passage 25) were grown to confluency as described in Table 1. The plates were washed with Hanks balanced salt solution. In experiment a, 8 ml WME containing 0.5% calf serum was added to the plates for 24 hr prior to addition of WME (no additions) or WME containing DMSO or PMA and incubated for a further 24 hr. In experiment b, 8 ml WME containing 1% BSA was added to the cells for 24 hr. Then either WME alone or WME containing dioctanoyl glycerol was added and the cells harvested 24 hr later. The values are reported as means ± SEM. $n = 4$ plates for each group. None of the differences were significant ($P > 0.2$).

the possibility that serum growth factors may play a mediating role in the transport response (Prasad, Severini & Kaplan, 1987; Grinstein et al., 1988). Conversely, incubation of ARL_{15} cells in low K^+ medium evoked a 25% increase in DNA content, a 100% increase in RNA content and a 25% increase in protein content after 24 hr (Pressley et al., 1988). The linkage between the ion transport and growth responses raised the possibility that serum growth factors may play a mediating role in the response to low K^+.

The present results indicate that Na,K-ATPase activity of the ARL_{15} system is insensitive to the presence of serum in the medium and that serum factors are not required for induction of Na,K-ATPase by low K^+.

The magnitudes of the Na,K-ATPase increases in activity (30–57%) in response to low K^+ in the absence and presence of serum were indistinguishable. Previous studies on low K^+ induction of Na,K-ATPase activity in ARL_{15} cells were carried out in the presence of 5% serum (Pressley et al., 1986; Ismail-Beigi et al., 1988; Pressley et al., 1988). The possibility that residual serum may have remained associated with the cells in the present study, of course, does exist. However, extensive washing during changes in medium and the extended period of exposure to serum depletion (48 hr) suggest that serum growth factors are unlikely to be cofactors in the response to low K^+, in that significant amounts of serum are usually required to elicit growth responses in culture (Gospodarowicz & Moran, 1974).

The insensitivity to serum of the Na,K-ATPase system in ARL_{15} cells is a specific trait in this cell line since another rat liver-derived cell line, clone 9 cells, exhibits increases in active ion fluxes, as well as in Na,K-ATPase activity in response to serum (Bhutada et al., 1990). The basis for this discrepancy (ARL_{15} *vs.* clone 9 cells) has yet to be elucidated.

In the presence of 5% calf serum, exposure to low K^+ elicits a "growth" response, in addition to induction of Na,K-ATPase, as evidenced by 25% increases in protein and DNA content, and a 100% increase in RNA content in ARL_{15} cells (Pressley et al., 1988). The serum independence of the response to low K^+ in ARL_{15} cells raises the possibility that the obligatory distortions in ion composition activate some component of the pathways that mediate the responses to serum growth factors, independent of the presence of these factors in the medium. To evaluate this possibility, we chose four proto-oncogenes as references; *c-fos, c-myc, c-jun* and *c-ski*. *c-fos* and *c-myc* are early growth response genes, and *c-jun* encodes a product that with *c-fos* is a part of the AP-1 transcription complex (Curran et al., 1985; Bishop, 1987; Lau & Nathans, 1987; Chiu et al., 1988). *c-ski* has been implicated in muscle differentiation and was used here as an index of possible generalized effect (Colmenares & Stavnezer, 1989).

Incubation of ARL_{15} cells in low K^+ elicited an early (30-min) transient increase in the abundance of *c-fos* mRNA. Since this occurred before the first detectable increases in the abundances of the Na,K-ATPase α and β subunit mRNAs (Pressley et al., 1988), the possibility that *c-fos* is part of a central

pathway that can be elicited either by growth factors or by low K^+ must be considered.

Induction of $mRNA_{fos}$ by low K^+ was transient, with the peak at 30 min and disappearance at 1 hr. In a variety of cell lines, the mitogenic response to growth factors is also accompanied by transient induction of $mRNA_{c\text{-}fos}$, with a very similar time course (Curran et al., 1985; Thompson et al., 1985). Accordingly, our findings are consistent with induction of a "growth-like" pathway by low K^+.

The results obtained on *c-myc*, *c-jun* and *c-ski* differed from that on *c-fos* in two respects: (i) mRNAs of *c-jun*, *c-myc* and *c-ski* were detected at fairly constant amounts for up to 2 hr in control cultures even when growth arrested by serum deprivation and by having attained confluency. These results imply constitutive expression in contrast to *c-fos*. (ii) Incubation in low K^+ in serum-free media had no significant effect on the abundances of these mRNAs (*c-jun*, *c-myc* and *c-ski*). The presence of detectable levels of *c-jun* and *c-fos* mRNAs have also been reported in "quiescent" hepatocyte cultures (Kruijer et al., 1986). As constitutive expression of the *c-myc*, *c-ski* and *c-jun* mRNAs were somewhat variable, small changes in their expression elicited by low K^+ may have been masked. This could explain why the 40 and 70% relative increases of low K^+ *versus* normal K^+ for *c-jun* at 30 min and for *c-ski* at 120 min were not statistically significant. On the other hand, with *c-fos*, there was no background and the relative increase of low K^+ *versus* normal K^+ of 290% was highly significant.

The invariance of mRNA-*myc* in low K^+ in ARL_{15} cells is in contrast to the commonly reported sequential induction of *c-fos* and *c-myc* by growth factors (Kelly et al., 1983; Lau & Nathans, 1987). If *c-fos* is a key intermediate in the response to low K^+, *c-fos* may turn on some cell functions without utilizing *c-myc* as an intermediate or in combination with constitutively expressed *c-myc*. The invariance in *c-jun* RNA is also of interest in view of the conjoint relationship with *c-fos* in the AP-1 transcription factor complex (Lau & Nathans, 1987). Further studies are needed to determine whether constitutive expression of *c-jun* (or *c-myc*) play a permissive role in the response to low K^+.

During low K^+ treatment there is a net loss of intracellular K^+ and a net gain of intracellular Na^+ (Graves & Wheeler, 1982; Kim et al., 1984; Pressley et al., 1986). Hence the present findings are consistent with the finding that shows Na^+ deprivation inhibits induction of *c-fos* expression by epidermal growth factor in cultured hepatocytes (Kuijer et al., 1986). Further evidence that oncogenes are regulated by ion levels is the induction of *c-fos* and *c-myc* by the Ca^{2+} ionophore A23187, implying a regulatory role for extracellular Ca^{2+} concentrations (Moore et al., 1986). Growth factor induced ion fluxes have been implicated in the regulation of expression of *c-fos* and *c-myc* in thymocytes and neurons (Moore et al., 1986; Morgan & Curran, 1986). Conversely, phorbol esters stimulated the Na/K pump in pancreatic acinar cells and hepatocytes, the electroneutral Na^+/H^+ antiporter in thymocytes and the Na^+/Ca^{2+} antiporter in aortic smooth muscle cells (Grinstein & Rothstein, 1986; Lynch et al., 1986; Grinstein et al., 1988; Vigne et al., 1988).

If transient induction of *c-fos* is a mediator of the response to low K^+, induction by an alternate route of equivalent magnitude and time course should induce Na,K-ATPase activity to an equivalent degree. To test this possibility we chose two well-characterized stimulators (PMA and dioctanoyl glycerol) of protein kinase C that induce *c-fos* transiently in a variety of cell culture systems (Nishizuka, 1986). PMA (93.7 ng/ml) or dioctanoyl glycerol (200 μM) had no significant effect on the Na,K-ATPase activity, although PMA stimulated *c-fos* mRNA levels to as great or greater extent than low K^+. These results seem to exclude induction of *c-fos* as a unique mediator.

PMA and dioctanoyl glycerol may have exerted effects on Na,K-ATPase activity at time periods other than those used here. Lynch et al. (1986) found that PMA and mezerein, another tumor promoter, stimulated Na,K-ATPase mediated transport activity of hepatic cells, presumably via activation of protein kinase C, in cell suspensions 5 min after challenge. Similarly, increased Na/K pump activity was obtained with TPA and the diacylglycerol analogue, 1-oleoyl-2-acetoyl-sn-3-glycerol in hepatic acinar cell suspensions within 60 min (Hootman, Brown & Williams, 1987). In contrast, in retinal capillary endothelial cells (Lee et al., 1989), rat liver slices (Smart & Deth, 1988), synaptosomal membranes (Oishi et al., 1988), and in kidney proximal tubule segments (Bertorello & Aperia, 1989), PMA decreased Na,K-ATPase activity. These results raise the possibility of activation of multiple pathways that may be either excitatory or inhibitory with PMA.

Although induction of *c-fos* was not sufficient to augment Na,K-ATPase activity in ARL_{15} cells, it may be a necessary cofactor in the response. Such a permissive role for *c-fos* can be tested by blocking its expression by transfection of cells with inducible *c-fos* antisense DNA prior to low K^+ challenge.

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