

**Membrane-mediated alterations of intracellular Na<sup>+</sup> and K<sup>+</sup>  
in lytic-virus-infected and retrovirus-transformed cells**

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*(Received 12 July 1982)*

Infection of chick-embryo fibroblasts and other cells by certain animal viruses results in alterations in the intracellular concentrations of Na<sup>+</sup> and K<sup>+</sup>. Dramatic alterations in monovalent-cation concentrations of lytic-virus-infected cells may favor the synthesis of viral proteins over cellular proteins. More subtle alterations in retrovirus-transformed cells may result in the expression of many morphological and biochemical changes associated with the transformed phenotype.

Elucidation of the mechanisms by which viruses influence cell-specified macromolecular synthesis is one of the most intensely pursued goals of molecular biology. Lytic viruses which induce the death of the host cell generally do so by subordination of host-specified macromolecular synthesis. Lytic viruses may have two effects on protein synthesis in the infected cell (1,2) which may not occur simultaneously. One effect is a reduction in the overall rate of protein synthesis, which may be nonselective. Virus- and cell-specified proteins are synthesized at a reduced rate in the infected cell. A second effect is a selective inhibition of cellular protein synthesis and a switchover from cell- to virus-specified protein synthesis. Both the decline in the overall rate of protein synthesis and the selective inhibition of host-specified protein synthesis appear to involve the initiation step of protein synthesis. Host mRNAs are not degraded in the lytic-virus-infected cell and are functional in *in vitro* protein-synthesizing systems (2-5). Host and viral mRNAs must have a distinguishing feature to account for the selective translation of viral mRNA in the infected cell. Discrimination between host and virus mRNA translation may involve an alteration in a component(s) of the initiation complex.

Transforming viruses, such as retroviruses, generally do not inhibit host macromolecular synthesis, but they induce the expression of a new set of phenotypic characteristics termed transformation parameters. Retroviruses contain genes, oncogenes, which are believed to encode proteins responsible for phenotypically altering the transformed cell (6). The mechanism by which these proteins induce cell transformation is unknown. Results summarized here suggest that both lytic and transforming viruses may employ a common strategy, alteration of the intracellular Na<sup>+</sup> and K<sup>+</sup> concentrations, to affect cellular gene expression.

## Methods

Methods for measurement of cell- and virus-specified macromolecular synthesis, determination of intracellular  $\text{Na}^+$  and  $\text{K}^+$  concentrations, and determination of host polysome profiles in chick-embryo fibroblasts and BHK cells have been described previously (7-9). Interferon assays and assays of the levels of antiviral proteins have also been described (8). The evaluation of retrovirus-induced transformation parameters was performed as detailed in Garry et al. (10).

## Results and Discussion

### *Effect of altered $\text{Na}^+$ media on cell- and virus-specified protein synthesis*

The intracellular concentration of  $\text{Na}^+$  is low and the intracellular concentration of  $\text{K}^+$  is high relative to the concentrations in the extracellular medium (11). Koch and colleagues (12) demonstrated that incubation of uninfected cells in medium containing elevated concentrations of  $\text{Na}^+$  (among other treatments) inhibits the initiation of protein synthesis. Initiation of translation of cellular proteins is also inhibited by incubating cells in media containing decreased concentrations of  $\text{Na}^+$  (7,8). Initiation of translation of cellular mRNAs is inhibited because incubation in high- $\text{Na}^+$  medium results in an increase in intracellular  $\text{Na}^+$  and incubation in low- $\text{Na}^+$  medium results in a decrease in intracellular  $\text{K}^+$  (7). Protein synthesis specified by a variety of different viruses is much more resistant than cell-specified protein synthesis to the initiation block induced by altered  $\text{Na}^+$  medium (7-15). Virus-specified mRNAs are translated more efficiently than cellular mRNAs across a wider range of monovalent-cation concentration in vitro.  $\text{Na}^+$  is increased and the concentration of  $\text{K}^+$  decreased by treatment with ouabain, which inhibits the membrane-associated ( $\text{Na}^+/\text{K}^+$ )ATPase (9). Consequently, ouabain treatment inhibits cellular protein synthesis, but is less inhibitory to protein synthesis specified by certain viruses (9,16).

Not all cell mRNAs are inhibited equally by lytic virus infection (Table 1). Nuss and Koch (17) showed that compared to other cellular proteins the synthesis of IgG in myeloma cells was much more resistant to the selective inhibition of cellular protein synthesis induced by vesicular stomatitis virus (VSV) infection. Interferon and certain heat-shock proteins are induced during lytic virus infections (8,18; Garry, Niesel, and Bose, submitted). An excellent correlation exists between the ability of a cellular protein to be synthesized during lytic virus infection and in cells with altered intracellular concentrations of  $\text{Na}^+$  and  $\text{K}^+$ . The synthesis of IgG is much more resistant to hypertonic medium than the synthesis of most cellular proteins (19). Fibroblast interferon synthesis, but not synthesis of the interferon-induced antiviral proteins, is as resistant as the synthesis of virus-specified proteins to altered  $\text{Na}^+$  media (8) and to ouabain (20,21). The synthesis of heat-shock proteins is also resistant to altered  $\text{Na}^+$  media (22; Garry, Niesel, and Bose, submitted). Differential sensitivity of the translation of certain lens proteins may play an important role in the formation of cataracts (23).

Table 1. Synthesis of cellular proteins under conditions which alter intracellular Na<sup>+</sup> and K<sup>+</sup>

Numbers in parentheses are reference citations.

Protein	Lytic virus infection	Altered Na <sup>+</sup> medium	Ouabain
IgG	resistant (17)	resistant (19)	not tested
Interferon	resistant (8)	resistant (8)	resistant (20,21)
Antiviral protein	sensitive (8)	sensitive (8)	sensitive (20,21)
Heat-shock proteins	resistant (22;*)	resistant (22;*)	not tested
High-mol.-wt. $\delta$ -crystallin	not tested	not tested	resistant (23)
Low-mol.-wt. $\delta$ -crystallin	not tested	not tested	sensitive (23)

\*Garry et al., submitted.

*Effect of lytic viruses on intracellular Na<sup>+</sup> and K<sup>+</sup> concentrations*

In cells lytically infected with Sindbis virus (SB), an alphavirus, or VSV, a rhabdovirus, the intracellular Na<sup>+</sup> concentration increases (from 20 mM to over 60 mM) while the intracellular concentration of K<sup>+</sup> decreases (from 150 mM to under 60 mM) (Table 2) (7,8). Alterations of intracellular ion concentrations also develop in cells infected by encephalomyocarditis virus (EMC), a picornavirus (Table 2) (24). The changes in intracellular ion concentrations found in these lytic-virus-infected cells are of sufficient magnitude to inhibit the translation of most host mRNAs. Translation of virus-specified mRNAs is unaffected or even stimulated by these changes. In SB- and VSV-infected cells the alteration in intracellular ion concentration, the reduction in the overall rate of protein synthesis, and the selective inhibition of host protein synthesis occur simultaneously. In EMC-infected cells an alteration in intracellular ion concentration also occurs simultaneously with the overall reduction in the rate of protein synthesis. The selective inhibition of host protein synthesis in EMC-infected cells, however, occurs later in infection at a time when viral mRNAs accumulate. Manipulating the concentration of salts in the medium, which presumably restores the intracellular concentration of Na<sup>+</sup> to the levels found in uninfected cells, reverses the selective inhibition of cell-specified protein synthesis in EMC-infected cells (25). These results suggest that the reduction in overall rate of protein synthesis observed in cells infected with SB, VSV, and EMC may be the result of the alteration in the intracellular ion concentrations. Alterations in the intracellular ion concentrations may also be required to selectively inhibit host-specified protein synthesis. This effect requires the accumulation of viral mRNAs which may compete

Table 2. Effects of lytic RNA-virus infections on intracellular  $\text{Na}^+$  and  $\text{K}^+$  levels and protein synthesis

Numbers in parentheses are reference citations.

Virus/cell	Alteration in intracellular $\text{Na}^+$ and/or $\text{K}^+$ levels	Decline in overall rate of protein synthesis	Selective inhibition of host protein synthesis
Sindbis virus/CE cells (9)	2 h	2 h	2 h
Vesicular stomatitis virus/CE cells (8)	3 h	3 h	3 h
Encephalomyocarditis virus/L cells (24)	3 h	3 h	4 h

with the host mRNAs for a protein synthesis factor that is sensitive to the altered ionic environment. Alterations in the intracellular concentrations of  $\text{Na}^+$  and  $\text{K}^+$  have also been documented in cells infected with SV40 (26), adenovirus (5), and, at a late stage of infection, with poliovirus (27; A. Schaefer, R. Zibirre, P. Kabus, J. Kuhne, and G. Koch, personal communication, and ref. 28), but may not occur during infections by other viruses (29).

*Effect of Sindbis virus infection on monovalent cation transport*

Carrasco (30) proposed that an increase in the permeability of lytic-virus-infected cells to  $\text{Na}^+$  results in an increase in intracellular  $\text{Na}^+$  and a selective termination of host protein synthesis. Intracellular concentrations of  $\text{Na}^+$  and  $\text{K}^+$  are maintained by a number of active and passive ion-transport systems (11,31). We have examined ion transport and permeability in cells infected by Sindbis virus (Ulug and Bose, in preparation). Sindbis virus infection results in a 40% inhibition of the membrane-associated  $(\text{Na}^+/\text{K}^+)\text{ATPase}$  (sodium pump) activity as determined by ouabain-sensitive influx experiments utilizing the  $\text{K}^+$  tracer  $^{86}\text{Rb}$ . The furosemide-sensitive  $\text{Na}^+/\text{K}^+/\text{Cl}^-$  cotransport system (31) is not inhibited during Sindbis virus infection. Further evidence that the  $(\text{Na}^+/\text{K}^+)\text{ATPase}$  is inhibited is provided by a parallel decrease in the binding of [ $^3\text{H}$ ]ouabain to SB-infected cells. Likewise, there is a substantial decrease in the  $(\text{Na}^+/\text{K}^+)\text{ATPase}$  activity of plasma membrane fractions obtained from SB-infected cells. Moreover, we find no evidence for increased permeability or leakage of monovalent ions. The changes in  $(\text{Na}^+/\text{K}^+)\text{ATPase}$  activity are temporally correlated with the selective inhibition of cellular protein synthesis. Thus, while the intracellular concentration of  $\text{Na}^+$  increases (and the concentration of  $\text{K}^+$  decreases) during Sindbis virus infection these changes are due to an inhibition of the  $(\text{Na}^+/\text{K}^+)\text{ATPase}$  activity and not to an increase in nonspecific membrane permeability.

*Na<sup>+</sup> and K<sup>+</sup> retrovirus transformation*

One of the earliest events after stimulation of lymphocytes by mitogens is the activation of the sodium pump (32). Nerve growth factor also activates the sodium pump (S. Varon, personal communication), and increased fluxes of monovalent ions are an early event in growth stimulation induced in cultured cells by the addition of serum (33,34). Transformation by retroviruses also results in alterations in the growth properties of animal cells. Chick-embryo (CE) cells transformed by the Bryan (B) and Schmidt-Ruppin (SR) strains of Rous sarcoma virus (RSV) exhibit strain-specific changes in their intracellular Na<sup>+</sup> and K<sup>+</sup> concentrations (10,35,36). The magnitudes of these changes are less or the directions are different from the changes in ion concentrations in lytic-virus-infected cells. Lowering the Na<sup>+</sup> concentration of the medium in which normal chick-cell cultures are incubated causes them to enlarge, vacuolate, and appear morphologically similar to cells transformed by B-RSV (10). Raising the Na<sup>+</sup> concentration of the medium causes cells to become round or spindle-shaped and to appear morphologically similar to cells transformed by SR-RSV. Uninfected chick-cell cultures incubated in either low- or high-Na<sup>+</sup> medium also express many other phenotypic characteristics of transformed cells. They lose contact inhibition of growth and movement, grow to higher saturation densities, synthesize reduced amounts of fibronectin, exhibit increased lectin agglutinability, transport increased amounts of hexoses, have reduced levels of succinic dehydrogenase activity, produce increased amounts of lactate and pyruvate, and can be serially passaged in culture significantly longer than normal chick fibroblasts. Chick cells incubated in altered Na media do not exhibit all transformation parameters expressed by RSV-transformed cells. The cells did not grow in soft agar or in low-serum medium. These results identify a possible new transformation parameter, alteration of the intracellular Na<sup>+</sup> and K<sup>+</sup> concentrations, and suggest that many, but not all, transformation parameters may be consequences of the altered intracellular concentrations of monovalent cations.

The alterations in intracellular Na<sup>+</sup> and K<sup>+</sup> induced by RSV are dependent on a functional transforming gene: *src*. The *src* gene product, pp60<sup>src</sup>, possesses a protein kinase activity with the unusual specificity for tyrosyl residues (6). The mechanism utilized by pp60<sup>src</sup> to alter intracellular ion concentrations is unknown. Several ion-transport systems, such as the (Na<sup>+</sup>/K<sup>+</sup>)ATPase, are known to be regulated by phosphorylation (5). In addition, Banerjee and coworkers (37) reported that the level of [<sup>3</sup>H]ouabain-binding sites decreases in cells transformed by SR-RSV. Bader et al. (36) reported that cells infected by B-RSV have the same number of [<sup>3</sup>H]ouabain-binding sites/mg protein as control cells. Since B-RSV-transformed CE cells are larger and contain more protein than untransformed cells (38), the number of [<sup>3</sup>H]ouabain-binding sites per cell is higher in the B-RSV-transformed CE cells.

**Acknowledgements**

This work was supported by Public Health Service Grants CA 26169

and CA 27003. R.F.G. was a postdoctoral fellow of the National Cancer Institute (CA 06593).

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