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Articles

Effect of Medium Hypertonicity on Reovirus Translation Rates. An Application of Kinetic Modeling in Vivo†

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ABSTRACT: Translation rates were determined for host and virus mRNAs in reovirus-infected SC-1 cells in hypertonic medium. The effect of low doses of cycloheximide on these translation rates was also measured. The results show that hypertonicity selectively stimulates viral translation relative to host translation. Moreover, in hypertonic medium, host translation is slightly stimulated by low doses of cycloheximide, whereas viral translation is markedly inhibited. This effect of cycloheximide is precisely the opposite to what was previously observed in isotonic media [Walden, W. E., Godefroy-Colburn, T., & Thach, R. E. (1981) *J. Biol. Chem. 256*, 11739–11746]. It is shown that both these effects of hypertonicity are predicted by the message competition/discrimination model previously described and thus provide support for the applicability of certain aspects of the model to translation rates in vivo.

Over the past 10 years, considerable evidence has accrued that suggests that message discriminatory initiation factors, in addition to 40S ribosomes, can influence the individual initiation rate for different types of mRNA (Lodish, 1974; Golini et al., 1976; Kabat & Chappell, 1977; DiSegni et al., 1979; Gette & Heywood, 1979; Heywood & Kennedy, 1979; Herson et al., 1979; Parets-Soler et al., 1981; Rosen et al., 1982; Ray et al., 1983; Sarkar et al., 1984; Godefroy-Colburn et al., 1985). It has been suggested that in vivo the concentration of mRNA is higher than that of one such discriminatory factor: thus, mRNAs with the highest affinities for factor outcompete those with lower affinities, and thereby are translated at higher rates (Walden et al., 1981; Godefroy-Colburn & Thach, 1981). While this theory, which we call the "message competition/discrimination (CD)[1] model", is consistent with a large amount of experimental data, further applications and tests of its accuracy in vivo are desirable.

Such an applicability test of the CD model is described in this paper. This is based on the translational response of

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[1] Abbreviations: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; eIF, eukaryotic initiation factor; TCA, trichloroacetic acid; R*, the 43S ribosomal complex; M*, the complex between mRNA and discriminatory factor; CD, competition/discrimination; MEM, minimal essential medium.

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different mRNAs to salt concentration. Reoviral mRNAs are poorer competitors than host mRNAs for in vitro translation at low salt concentrations (Brendler et al., 1981a,b). Now, if the same set of relationships holds true in vivo, then the CD model predicts that cycloheximide should stimulate the rate of translation of reoviral mRNAs whereas that of host mRNAs should be inhibited. This stimulatory effect of cycloheximide on reovirus translation has been observed in infected cells in isotonic medium, as has already been reported (Walden et al., 1981). By contrast, reoviral mRNAs are much stronger competitors than host mRNAs for in vitro translation at high salt concentrations (Brendler et al., 1981a,b). Thus, the model further predicts that if the in vivo salt concentrations could be substantially increased, then a reversal of the cycloheximide specificity should occur: now translation of reovirus mRNAs should be inhibited, while that of host mRNAs should be stimulated, by cycloheximide.

Following this logic, we have studied the effects of ionic environment on translation rates in reovirus-infected cells. Ionic variation was achieved by adding sucrose or NaCl to the cell culture medium to produce a hypertonic milieu. It has been shown by several investigators that medium hypertonicity can have important differential effects on translation rates (Koch et al., 1976; Alonso & Carrasco, 1981). Some of these differential effects can be reproduced in vitro by varying the ionic conditions, suggesting that the hypertonic effect may simply be due to the cumulative effects of ions on the different steps in the translation pathway (Alonso & Carrasco, 1981). Thus, it seemed possible that this procedure might be useful in looking for the predicted reversal of cycloheximide sensitivities in reovirus-infected cells.

The experimental data obtained in this study were also analyzed quantitatively through the use of a mathematical algorithm of the CD model (Godefroy-Colburn & Thach, 1981). This algorithm was developed by adding to the earlier model of Lodish (1974) a step in which excess mRNAs compete for binding to a limiting discriminatory factor. In addition, provision for the scanning of the message by a bound 40S ribosomal subunit (Kozak, 1978) was included, as was a set of terms describing the conservation of ribosomal subunits. Through the use of this algorithm it could be determined whether the tonicity-induced changes seen in vivo were quantitatively consistent with the salt-related changes previously measured in vitro.

The results of this study show that the predicted reversal of cycloheximide sensitivities does in fact occur when reovirus-infected cells are placed in hypertonic medium. Moreover, the results are quantitatively consistent with comparable measurements made in vitro.

Materials and Methods

Cell Growth, Virus Infection, Labeling, and Analysis of Proteins. All procedures were as previously described (Walden et al., 1981), except that cells were grown in 4% instead of 2% fetal calf serum. In brief, mouse SC-1 fibroblasts were seeded at 0.25×10^4 per cm^2 in Costar 6 well Petri plates in McCoy's 5A medium supplemented with 10% fetal calf serum (Irvine Scientific). One day later, 2.0 mL of fresh culture medium containing 4% fetal calf serum was added per well. On the following day, cells were infected with reovirus. Eight hours later, MEM medium containing one-tenth the normal amount of methionine, plus amounts of cycloheximide and/or sucrose as indicated in the figure legends, was added. After a 15-min equilibration period, this medium was replaced with 0.5 mL of identical medium supplemented with 50 μCi of [^{35}S]methionine. After a 30-min labeling period, this medium was replaced with identical medium, but now containing 1 mM unlabeled methionine instead of [^{35}S]methionine. After a 15-min chase period, proteins were precipitated with cold TCA, resuspended in electrophoresis sample buffer, and analyzed by SDS–PAGE according to the method of Laemmli (1970). Autoradiograms of the dried gels were quantitated by scanning with a Joyce-Loebl densitometer.

It is important to note that, under the conditions employed, i.e., early in the infection of SC-1 cells, host translation is only slightly inhibited and the majority of the reovirus mRNAs in the cell are capped (Detjen et al., 1981; Zarbl & Millward, 1983). Substantial amounts of uncapped reoviral mRNAs have only been reported in L-cells late in the infectious cycle (Skup et al., 1981).

Cell Volume and Internal Potassium Measurements. Average cell volumes for infected and control cells were measured in a Coulter Counter, or by the hematocrit method. Both methods gave similar results. Internal potassium levels were determined by flame photometry as previously described (Ramabhadran & Thach, 1981).

Identification and Quantitation of Reovirus Proteins in Polyacrylamide Gel Electrophoresis. Study of reoviral translational controls has been made difficult by problems in identifying the various gene products in SDS–PAGE gels. The electrophoretic mobilities of several viral proteins are variable and depend strongly on experimental conditions (McRae & Joklik, 1978; Mustoe et al., 1978; Samuel, 1983). An example of this variability is evident upon comparison of Figures 3 and 6. While the conditions of electrophoresis in the two experiments shown varied only slightly, the relative migration rates of σ_{NS}, σ_3, and σ_2 were different. In our hands, σ_{NS} always migrates close to σ_3 and usually overlaps it. This is true of both in vivo products and the in vitro products obtained by translating purified S_3 and S_4 dsRNA (Brendler et al., 1981a). Because of the difficulty in resolving σ_3 and σ_{NS} we have combined the data for these two proteins in some figures. This does not seriously affect our results or conclusions since the kinetic parameters (Brendler et al., 1981a) and the message transcription rates in vivo (Zweerink & Joklik, 1970) for the two are quite similar. Moreover, translation rates of both proteins responded very similarly to hypertonicity, as described in the text. The product of the L_1 gene, λ_3, and the two small S-class products, σ_1 and σ_2, were resolvable in most gels but were not produced in sufficient quantities to allow reliable quantitation. However, in many cases qualitative judgments as to the behavior of these proteins are possible. Again, this presents no problem for the kinetic analysis, since each message was modeled independently. The only message for which we have no data is M_1, which encodes μ_2. However, since this represents less than 1% of the viral products, it has been ignored.

In spite of these difficulties, quantitation of the rates of synthesis of several viral proteins was accurate and reproducible. This was primarily due to the fact that within a given electrophoretogram host protein migration was highly reproducible from lane to lane. Thus, precise background values could usually be determined by densitometric scanning of control lanes and subtracted from viral peaks.

Kinetic Modeling. The algorithm previously described (Godefroy-Colburn & Thach, 1981) was employed. Solutions were obtained by a standard reiterative technique by using an IBM personal computer programmed in Advanced Basic (copies available upon request). Eleven mRNAs were modeled, with name of encoded protein, numerical designation, codon length, and concentration as follows: average host, 1,

350, 190 nM; actin, 2, 425, 12 nM; reoviral λ_1, 3, 1390, 0.5 nM; reoviral λ_2, 4, 1345, 0.5 nM; reoviral λ_3, 5, 1300, 0.5 nM; reoviral μ_1, 6, 718, 3 nM; reoviral μ_{NS}, 7, 655, 5.1 nM; reoviral σ_1, 8, 409, 3.1 nM; reoviral σ_2, 9, 400, 5.1 nM; reoviral σ_{NS}, 10, 391, 10.1 nM; reoviral σ_3, 11, 382, 10.1 nM. The total concentration of all reovirus mRNAs was 40 nM, as determined previously (Walden et al., 1981; Godefroy-Colburn & Thach, 1981). The relative concentrations of individual viral mRNAs were calculated according to the transcriptional frequencies determined by Zweerink & Joklik (1970), except for that of reoviral σ_1: the relative concentration of its mRNA, S_1, has recently been revised downward by 40%.[2] The values of most other constants employed did not differ significantly from those used previously: $k_c = 54$ min^{-1}; $k_j = 1$ min^{-1} nM^{-1}; $K_1 = 50$ nM; $K_v = 500$ nM; $r_t = 4600$ nM; $f_t = 95$ nM; $e_t = 280$ nM; $L = 15$.

Recent evidence bears on the question as to whether the rate of scanning of the 5′ leader region of an mRNA, represented by the rate constant k_c, can limit the overall rate of translation. Kozak (1984) and Johansen et al. (1985) have recently concluded that the translation rate of most messages appears to be independent of the length of the 5′ leader region that must be scanned by a 40S ribosome. In contrast, it is clear that the introduction of very stable hairpin loops into the 5′ leader region can inhibit initiation (Pelletier & Sonenberg, 1985). Antczak et al. (1982) have examined the leader regions of all the reovirus mRNAs and found no possibilities for the formation of unusually stable structures. Thus, it seems that our use of a single value for k_c is justified for the mRNAs investigated here, at least under isotonic conditions (see Discussion).

The values for dissociation constants of discriminatory factor/mRNA complexes were obtained from the competition curves shown in Brendler et al. (1981a,b). The "Q'-plot" method described therein was first used to obtain "discrimination ratios", which were then normalized to a value of 1.0 for mouse L-cell mRNA. "Relative affinity" values were the reciprocals of these normalized discrimination ratios. The relative affinities of reovirus mRNAs for discriminatory factor are plotted in Figure 1 as functions of the Mg^{2+} and K^+ concentrations in the reaction mixtures. The spacing of entries along the abscissa was chosen so as to linearize the data points. This method of presenting the data reveals that the response of each mRNA to increasing salt concentration is both regular (i.e., monotonic) and unique and therefore must be dependent at least to some extent on its base sequence. To obtain dissociation constants (K_f^i), normalized discrimination ratios were multiplied by 10^{-9} M, a value which best fit the experimental data. (For example, at 60 mM KCl and 2.0 mM $Mg(OAc)_2$, $K_f^1 = 1.0$ nM, $K_f^7 = 1.61$ nM, $K_f^{11} = 2.38$ nM, etc.)

The values for apparent dissociation constants of R*/mRNA complexes were obtained from studies of the salt dependence on translation under conditions of mRNA saturation, shown in Figures 1 and 2 of Brendler et al. (1981b). Under these conditions the rate-limiting step for translation is the binding of message/discriminatory factor complexes, called M*, to R* (Lodish, 1974). Since the amino acid incorporation levels shown in the cited figures were directly proportional to translation rates, then these incorporation levels must also be proportional to the respective binding constants. Thus, the relative binding constants of M* to R* could be calculated directly from these published data. For purposes of mathematical modeling, we have chosen to interpret these

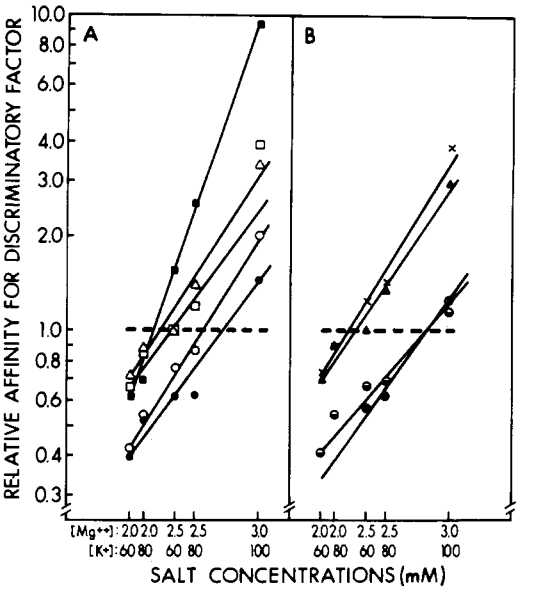

FIGURE 1: Effect of salt concentrations on the relative affinity between discriminatory factor and different mRNAs in vitro. Half-saturating concentrations of reoviral or mouse L cell mRNAs were translated in a fractionated system derived from Krebs ascites tumors, in the presence of varying amounts of globin mRNA, $Mg(OAc)_2$, and KCl. From the degree of translation inhibition by competing globin mRNA the relative affinities were calculated (see Materials and Methods). These were then normalized with respect to the values for mouse L-cell mRNA and plotted as a function of Mg^{2+} and K^+ concentrations in the reaction mixtures. Solid lines were drawn to fit data points for the following reoviral messages (indicated by the proteins they encode): (△) λ_1; (▲) λ_2; (×) λ_3; (□) μ_1; (■) μ_{NS}; (⊖) σ_1; (●) σ_2; (⊗) σ_{NS}; (○) σ_3. Dashed lines indicate the (normalized) value for mouse L-cell mRNA. Curves were presented in two panels, A and B, for convenience.

binding constants as being equivalent to equilibrium affinities between R* and M* complexes. However, as explained in the text, this interpretation may not be strictly correct at high salt concentrations, where the rate of R* binding may be influenced by such factors as message unwinding (Ray et al., 1985). To reflect this uncertainty, we shall refer to the constants used as "apparent affinities". Normalized affinities were obtained by setting the incorporation stimulated by mouse L-cell mRNA at 80 mM KCl and 2.5 mM $Mg(OAc)_2$ to a value of 1.0; these normalized apparent affinities are plotted in Figure 2, as a function of $Mg(OAc)_2$ concentration. The same value was used for all reovirus mRNAs at a given $Mg(OAc)_2$ concentration, as little or no difference among them was observed (Ray et al., 1983). Also, little variation of this parameter was observed with varying KCl concentration [see Figure 1 of Brendler et al. (1981b)]. To obtain apparent dissociation constants (K_r^i), the reciprocals of the values shown in Figure 2 were multiplied by 100 nM, a value which best fit the experimental data. (For example, at 60 mM KCl and 2.0 mM $Mg(OAc)_2$, $K_r^1 = 155$ nM, $K_r^7 = 151$ nM, $K_r^{11} = 151$ nM, etc.)

Finally, effects of salt concentrations on elongation rates were considered. Here the experimental data available were ambiguous. In vivo, a strong effect of K^+ on elongation rate was apparent at subnormal K^+ concentrations (Cahn & Lubin, 1978). However, at supranormal ion concentrations, little effect of increasing K^+ on elongation rate was observed (Saborio et al., 1974). By contrast, elongation rates in vitro were quite sensitive to K^+ concentration over a broad range, varying 1.4–1.6-fold per 50 mM increments of K^+ (Mathews & Osborn, 1974). As a compromise estimate, we have assumed that when isotonic medium is made hypertonic by the addition of

[2] Wolfgang K. Joklik, personal communication.

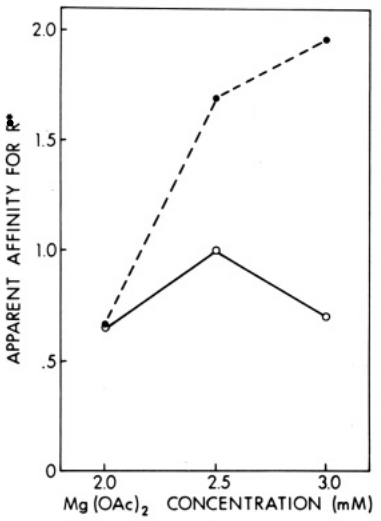

FIGURE 2: Effect of $Mg(OAc)_2$ concentration on the apparent relative affinity between 43S ribosomal complex and different mRNA/discriminatory factor complexes in vitro. Saturating concentrations of reoviral or mouse L-cell mRNAs were translated in a fractionated system derived from Krebs ascites tumors with varying concentrations of $Mg(OAc)_2$. From the overall rats of translation [shown in Figures 1 and 2 of Brendler et al. (1981b)] the apparent affinities of 43S complexes for different mRNA/discriminatory factor complexes were calculated as described under Materials and Methods. All values were normalized relative to a value of 1.0 for mouse L-cell mRNA at 2.5 mM $Mg(OAc)_2$ and plotted as a function of $Mg(OAc)_2$ concentration in the reaction mixtures. Dashed line, reoviral mRNA; solid line, mouse L-cell mRNAs.

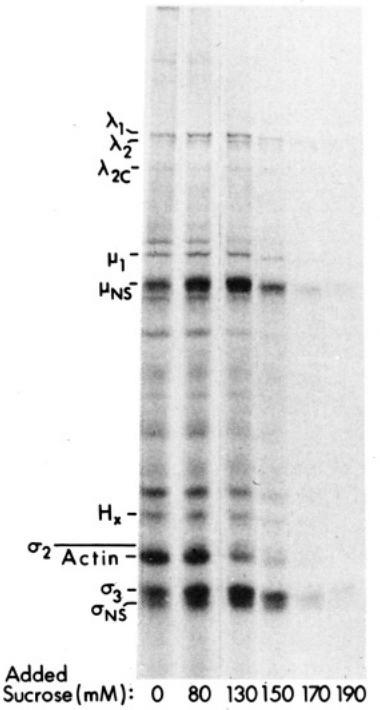

FIGURE 3: Effect of medium hypertonicity on reoviral and host translation rates in infected cells. Following a brief treatment with added sucrose as indicated, infected cells were pulse labeled with [^{35}S]methionine and chased with excess cold methionine, and the labeled proteins were then analyzed by SDS–PAGE and autoradiography, as described under Materials and Methods.

100 mM sucrose, the in vivo elongation rate constant, k_e, increases from 600 to 790 min^{-1}. In any event, the exact values used are not important for our purposes, since the modeling of experimental data is rather insensitive to increases in k_e.

Results

Effect of Medium Hypertonicity on Ion Concentrations in Reovirus-Infected and Control SC-1 Cells. Before the effects of medium hypertonicity on translation rates in reovirus-infected cells were considered, it was necessary to show that this treatment does in fact result in elevated intracellular ion concentrations. This was done in two ways. First, average cell volumes were measured for infected and control cells in normal and hypertonic medium (plus 100 mM sucrose). A median shrinkage of approximately 23% was induced by hypertonicity in both cell populations. From this we can conclude that internal solute concentrations do in fact rise with increased medium tonicity, in both control and reovirus-infected cells. In a second set of experiments, we investigated the possibility that while the overall internal osmotic strength may rise under these conditions, there might be an exchange of ions with the extracellular medium such that the internal ionic composition might change. In particular, we were concerned about an exchange of K^+ for Na^+ ions (Carrasco & Smith, 1976; Garry et al., 1979; Nair, 1981). To test this possibility, the amounts of intracellular K^+ were determined for infected and control cells in isotonic and hypertonic media. In neither case did a tonicity change cause any change in cellular K^+ content. This suggests that substantial changes in intracellular monovalent cation composition do not occur under the conditions employed.

Using these observations in conjunction with the Boyle–van't Hoff equation (Raaphorst & Kruuv, 1979), we were able to estimate the increase in the intracellular K^+ concentration caused by the addition of 100 mM sucrose to the medium. This increase was approximately 45% relative to the isotonic control. Inasmuch as the volumetric determinations indicate that both types of cells behave like osmometers, it seems likely that other internal ions rise in concentration by similar amounts.

Effect of Medium Hypertonicity on Reovirus Protein Synthesis in Vivo. The next step in this investigation was to determine the effect of hypertonicity on translation rates in reovirus-infected SC-1 cells. In most experiments of this kind sucrose was added to McCoy's 5A medium to adjust tonicity. A typical result showing the effect of added sucrose on reoviral and host translation rates is in Figure 3. Addition of up to 130 mM sucrose to the cell growth medium for 15 min prior to pulse labeling of proteins stimulates reoviral translation. This effect is most clear for λ_1, λ_2, μ_1, μ_{NS}, σ_3, and σ_{NS}. (Although the σ_3 and σ_{NS} bands are incompletely resolved, it is clear that they are both increased by added sucrose.) By contrast, translation of most host messages is not affected by 80 mM, and is slightly inhibited by 130 mM, sucrose (the message for host protein Hx is an exception). At higher sucrose concentrations translation of all messages is inhibited, although viral messages are more resistant than host. This latter effect has been noted by Nuss et al. (1976) in reovirus-infected L-cells. The specificity of the hypertonic effect is also evident in Figure 4A,B, where densitometric scans of lanes from an experiment similar to that in Figure 3 are shown. Here it is evident that addition of 110 mM sucrose to the growth medium more than doubles the synthesis rates of major reovirus proteins, whereas synthesis rates of most host proteins are unchanged. Similar results were obtained when NaCl was used to increase medium tonicity instead of sucrose (data not shown). Host translation in uninfected control cells responds similarly to that in infected cells to added sucrose, with little effect on most protein bands being observed until concentrations above 100 mM are reached (Figure 5).

These observations are consistent with the fact that reovirus mRNAs have higher competitive efficiencies than host mRNAs at high salt concentrations, and thus confirm one

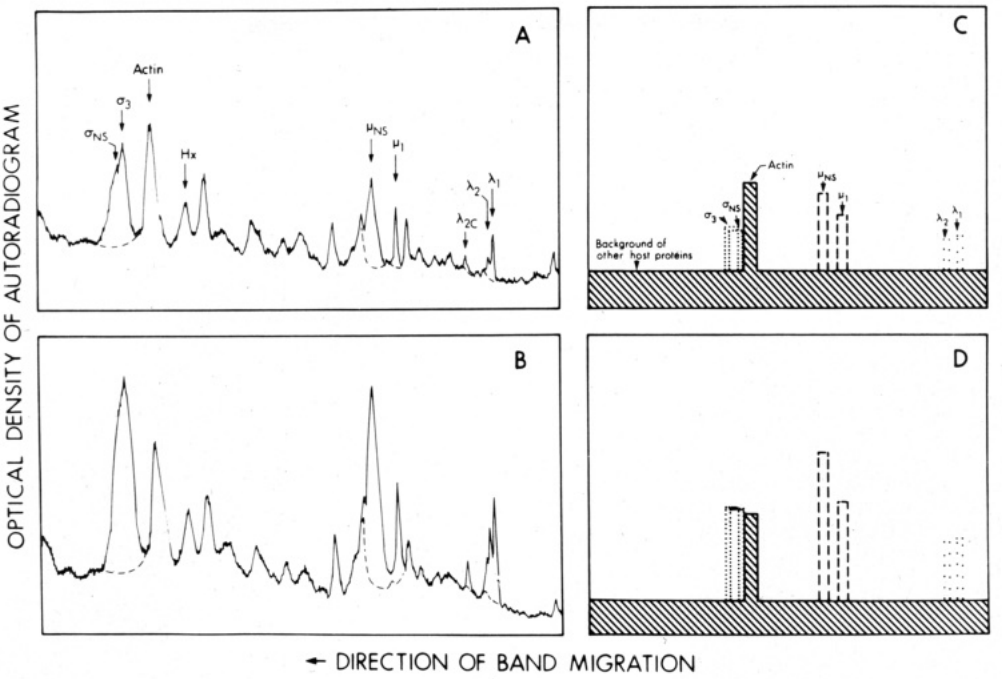

FIGURE 4: Effect of medium hypertonicity on reoviral and host translation rates: comparison of experimental and theoretical results. Densitometric scans of experiments similar to those in Figure 3 are shown. (Panel A) Synthesis of host and viral proteins in infected cells with no sucrose added to media; base lines for reoviral protein peaks determined from uninfected control cells are indicated by dashed lines. (Panel B) Synthesis of host and viral proteins in infected cells with 110 mM sucrose added to media. (Panel C) Simulation of scan in panel A using data produced by kinetic modeling; the area of each vertical bar is proportional to the rate of synthesis of the indicated protein; the area of the crosshatched horizontal bar is proportional to the synthesis of all host proteins other than actin. (Panel D) Simulation of scan in panel B using data produced by a kinetic model. See Materials and Methods for details.

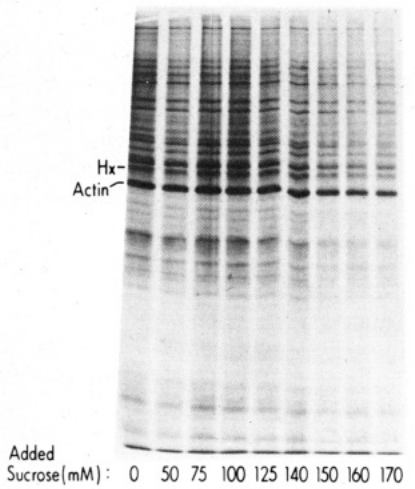

FIGURE 5: Effect of medium hypertonicity on host translation rates in uninfected cells. Following a brief treatment with added sucrose as indicated, uninfected cells were pulse labeled with [^{35}S]methionine and chased with excess cold methionine, and the labeled proteins were then analyzed by SDS–PAGE and autoradiography, as described under Materials and Methods.

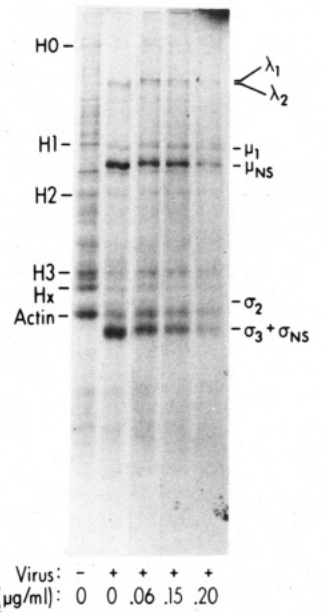

FIGURE 6: Effect of cycloheximide on reoviral and host translation in hypertonic media. Infected and control cells were treated briefly with 100 mM sucrose and cycloheximide as indicated, pulse labeled with [^{35}S]methionine, and chased with excess cold methionine, and the labeled proteins were then analyzed by SDS–PAGE and autoradiographed, as described under Materials and Methods.

prediction of the CD model (Brendler et al., 1981b). A more specific test of the model was obtained by using low concentrations of cycloheximide to further perturb the system. In isotonic media, brief exposure to low concentrations of cycloheximide has previously been shown to stimulate the synthesis of reoviral proteins (Walden et al., 1981). Now, in hypertonic medium we find that this cycloheximide effect is reversed. This result is shown in Figure 6 for cells pretreated with 100 mM sucrose. With the SDS–PAGE protocol employed in this experiment, the σ_3 and σ_{NS} bands were nearly coincident. However, better resolution of these bands was obtained in other experiments, from which it is clear that both σ_3 and σ_{NS} synthesis responded similarly to cycloheximide (data not shown). Quantitative measurements of synthesis rates were obtained by densitometry and are shown in Figure 7. It is evident from these data that reoviral translation is now more sensitive to cycloheximide than is host translation. Moreover, it is evident that synthesis of at least four host proteins is slightly stimulated by low doses of cycloheximide (Figure 7A). Although results for only two viral proteins are shown in Figure 7A, all viral proteins for which quantitative data could be obtained behaved similarly. This is evident in

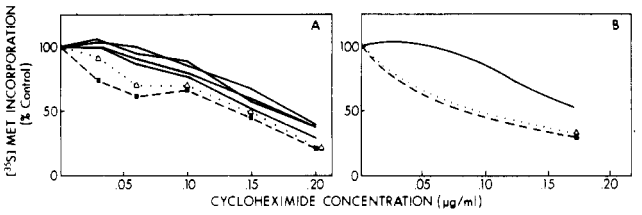

FIGURE 7: Differential effects of cycloheximide on reoviral and host translation in hypertonic media: comparison of experimental and theoretical results. (Panel A) Translation rates for host and viral messages (in the presence of 100 mM sucrose and the indicated concentrations of cycloheximide in the culture medium) were obtained by quantitation of densitometric scans of an autoradiogram similar to that shown in Figure 6; values were normalized by setting the zero cycloheximide values at 100%. Symbols for viral proteins are (■) μ_{NS} and (▲) λ_1. Host proteins chosen for quantitation (indicated in Figure 6) and represented by solid lines are H_1, H_2, H_3, and H_X. (Panel B) Simulation of results in panel A using data produced by kinetic modeling. Symbols are the same as in panel A, except that the solid line models the synthesis of a host protein comparable to actin.

Figure 8A–C, where the effect of cycloheximide treatment in hypertonic medium on the synthesis of λ_1, λ_2, μ_1, μ_{NS}, and σ_3 plus σ_{NS} is shown. (For ease of comparison the results have been normalized in Figure 8, with the synthesis of a typical host protein set at 100% for all cycloheximide concentrations.)

This reversal of the cycloheximide sensitivities of host and viral translation by hypertonicity is striking. It is also the key observation that indicates how hypertonicity affects translation rates in vivo, because it suggests that under these conditions reoviral mRNAs have become stronger competitors than host mRNAs. This is precisely the same effect seen with increasing ionic strength in vitro (Brendler et al., 1981b). The coincidence of these in vivo and in vitro results further supports the CD model. A quantitative analysis of the phenomenon provides additional insights, as is shown below.

Mimicking of in Vivo Results by Kinetic Modeling. The qualitative interpretation of the in vivo results described above seems reasonable. However, a more rigorous assessment could be made by analyzing the data quantitatively by using the kinetic algorithm previously described (Godefroy-Colburn & Thach, 1981). Before this could be done, the effects of salt concentrations on all the rate-determining steps in the translational pathway had to be evaluated. Three steps were selected as being the most important: (a) binding of discriminatory factor to mRNA; (b) binding of R* to mRNA; (c) movement of ribosomes along the mRNA during the elongation phase. The effects of salt concentration on each of these steps were determined as described under Materials and Methods. These three reactions may not be the only steps in the translation pathway that are differentially sensitive to ionic conditions in a message-specific manner. However, they do appear to be the ones that determine the overall rate of protein synthesis both in vivo and in vitro for most messages, and have been broadly implicated in other studies of translational control (Lodish, 1976; Ochoa & de Haro, 1979; Jagus et al., 1981; Walden & Thach, 1982). Hence, the data assembled here should be sufficient to describe the kinetic response of the entire pathway to ionic perturbation in vivo.

The three salt-sensitive in vitro parameters selected were then used to derive theoretical in vivo translation rates for individual reovirus and host mRNAs. All other parameters were assumed to be invariant with salt. The results of these calculations were then compared with the experimental data to see if the two were similar. This effort revealed that the parameters measured in vitro at the lowest salt concentrations tested could indeed be used to mimic the experimental data

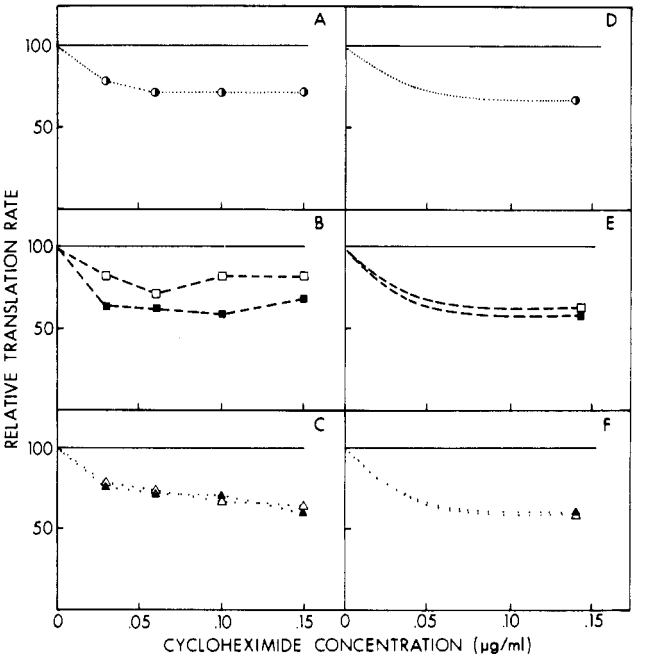

FIGURE 8: Differential effect of cycloheximide on translation of eight reoviral messages in hypertonic media: comparison of experimental and theoretical results. Translation rates for host and viral messages were obtained as described in Figure 5. Values for viral messages were normalized by setting the value for host protein H_0 (see Figure 6) at 100% at each concentration of cycloheximide. (Panels A–C) Experimental Results. (Panels D–F) Simulation of results in panels A–C by kinetic modeling. Symbols are (◑) $\sigma_3 + \sigma_{NS}$, (■) μ_{NS}, (□) μ_1, (△) λ_1, (▲) λ_2, and (—) H_0.

obtained in isotonic media. Conversely, in vitro parameters measured at the highest salt concentrations tested could produce results similar to those actually observed in the presence of 100 mM sucrose. Parameters intermediate between the two in vitro extremes fit experimental data obtained with intermediate concentrations of sucrose. A comparison of theoretical and experimental data is shown in Figure 4. Panel C of this figure is a bar graph arranged to mimic the polyacrylamide gel electrophoretogram shown in panel A. Peak areas, which are proportional to amino acid incorporation rates for the specified polypeptides, were calculated by using the lowest in vitro salt values. The correlation between the actual experimental results in panel A and the theoretical data in panel C is close. Similarly, the results in panel D were derived by using the highest salt in vitro values. Again, these data closely mimic the experimental results obtained with 100 mM sucrose shown in panel B.

The cycloheximide effects in hypertonic media could also be modeled accurately. This is evident in panel B of Figure 7, which mimics the experimental data shown in panel A. Of particular interest is the slight stimulation of host translation by cyloheximide. The effect of cycloheximide on all the reovirus bands studied was also reproduced faithfully by the model, as shown in Figure 8D–F.

Discussion

The results as described show that two predictions of the message competition/discrimination model are fulfilled in reovirus-infected cells. The first is that an increase in intracellular ion concentrations (produced by medium hypertonicity) should preferentially stimulate reovirus translation relative to host translation. The second is that under these conditions a reversal in cycloheximide sensitivities should occur: now host translation rates should be stimulated by low doses of cycloheximide, whereas reovirus translation should be in-

hibited. The fact that both of these peculiar effects are actually observed supports the applicability of the model in vivo.

We have considered the possibility that these two observations might be due to some other, totally different, effect of hypertonicity. Indeed, it is well-known that hypertonicity can cause a very complex set of intracellular changes, including viscosity, solute concentrations, membrane permeability, etc. (Mastro et al., 1984). However, by keeping the tonicity increase relatively small and the time of exposure short, we have eliminated some of these problems; moreover, others such as the increases in enzyme and substrate concentrations and viscosity can be shown to be unlikely contributors to the observed phenomena through mathematical modeling (unpublished results). Indeed, our results support the conclusions of others that ionic changes alone may be primarily responsible for the effects of hypertonicity on translation rates (Carrasco & Smith, 1976; Alonso & Carrasco, 1981). Moreover, they are consistent with the recent observation of R. Duncan and J. W. B. Hershey (personal communication) that hypertonicity does not result in covalent modifications of any known initiation factors.

As a prerequisite for the mathematical modeling studies, the salt dependence of three steps in the translational pathway was determined from previously published data. The most salt-sensitive step was the binding of mRNA to discriminatory factor. It is of particular interest that there were marked differences among the mRNAs studied in this respect (Figure 1). It is not clear at present why the binding of some mRNAs to discriminatory factor is more salt sensitive than that of others; however, it is evident that differences in base sequence must ultimately be responsible. We are currently investigating the hypothesis that it is the secondary and tertiary structures which result from sequence differences that are of primary importance in this reaction.

In addition to the discriminatory factor recognition step, our results are consistent with the existence of a second ion sensitive step that also influences translation initiation rates in vivo as well as in vitro. This is the binding of mRNA to the 43S ribosomal complex. This complex was called "R*" by Lodish (1974), who first proposed that it might interact differentially with mRNAs. That this step is salt dependent has been noted by several investigators (Weber et al., 1977; Bergmann & Lodish, 1979a; Sonenberg & Lee, 1982; Lee et al., 1983). In the present system, it is evident that the rate of reaction of reoviral messages with R* increases about 3-fold with increasing ionic strength, whereas that of host messages is less affected (Figure 2). In this reaction the mechanism by which salt exerts differential effects between reoviral and host mRNAs is not clear, and several possibilities must be considered. For example, the 3′-terminal poly(A) sequence present on most host mRNAs may play a role in this step. Since reovirus mRNAs lack poly(A) sequences, it is possible that this difference could contribute to the salt-dependent specificity of the R* interaction. Second, it may be that at high salt concentrations the scanning and/or unwinding of host mRNAs begins to become limiting (Pelletier & Sonenberg, 1985; Ray et al., 1985). Such effects, if present, would be included in changes in the apparent R* binding constant. Since reovirus mRNAs have very short leader sequences compared to host mRNAs (Kozak, 1984), they may be less susceptible to any such effects. Third, it is possible that phosphorylation of ribosomal proteins plays a role in the specificity changes observed in this step. Kruppa & Martini (1978) have shown that increased medium tonicity can induce the dephosphorylation of ribosomal protein S6 in vivo. Moreover, Burkhard & Traugh (1983) have recently observed differences in mRNA specificity that vary with the phosphorylation state of S6. Since the rate of S6 phosphorylation is also salt dependent in vitro, this phenomenon might contribute to the message specificity observed in the binding of R* to mRNAs reported here. Finally, it should be noted that the salt-sensitive discriminatory component of the 43S complex may not be the ribosome itself, but rather an initiation factor bound to it, such as eIF-2 or eIF-3. Indeed, Kaempfer and co-workers have shown that eIF-2 can discriminate among mRNAs, especially at supraoptimal salt concentrations (DiSegni et al., 1979; Rosen et al., 1982).

It is interesting to speculate on the biological significance of the response of reovirus translation to high salt concentrations. It seems unlikely that an infected cell would encounter hypertonic conditions in situ. Rather, a more plausible explanation is that this response is to specific ion imbalances that arise in cells late in the infectious cycle (Carrasco & Smith, 1976; Carrasco, 1977; Garry et al., 1979; Nair, 1981; Schaefer et al., 1983). In this view, then, the virus may have evolved the capacity to take advantage of intracellular conditions that it has created by unrelated processes.

The kinetic model used to compare in vitro and in vivo data is essentially the same as that described previously (Godefroy-Colburn & Thach, 1981). The success of this model in mimicking experimental results is satisfactory for most mRNAs. In itself, of course, this does not prove the correctness of every detail of the model, and it is clear that adjustments will have to be made as new facts come to light. For example, a new term must be added to reflect the fact that scanning ribosomes do not initiate at all AUG codons with the same frequency (Kozak, 1984; Liu et al., 1984; Ernst & Shatkin, 1985). One simple way to account for this effect would be to include a factor representing the initiation probability (where a value of 1.0 would correspond to a 5′-proximal AUG surrounded by a perfect consensus sequence) in the left most term of eq 3 in Colburn & Thach (1981). However, before such a modification can be undertaken, more quantitative data on the roles of neighboring sequences and multiple functioning AUG codons are required. In any event, these considerations do not effect the conclusions regarding mRNA competition and discrimination reported here.

The present study does not deal with the question of which initiation factor is the primary site of mRNA competition (termed "F" in the CD model). In previous studies it was suggested that this is likely to be eIF-4F, with the possibility of eIF-4A playing an ancillary role in some cases (Ray et al., 1983; Sarkar et al., 1984; Ray et al., 1985). In the present system, our modeling experiments suggest that the ratio of this factor to ribosomes must be on the order of 0.02. This is consistent with the recent observation that the ratio of eIF-4E to ribosomes is 0.15 in HeLa cells and 0.02 in reticulocytes (Duncan and Hershey, personal communication; Hiremath, Webb, and Rhoads, personal communication). These values for eIF-4E set an upper limit to the concentration of eIF-4F, which is much lower than that for other initiation factors but similar to our mathematically derived value. Moreover, it is evident that eIF-4A is unlikely to be the primary site of competition, as it is probably present in 20–150-fold higher quantities (Duncan & Hershey, 1983). By a similar argument, most other free initiation factors would seem to be unlikely candidates for this role.

Registry No. Cycloheximide, 66-81-9.

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