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Differential Translation in Normal and Adenovirus Type 5-Infected Human Cells and Cell-Free Systems

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When uninfected or adenovirus 5-infected KB cells are exposed to hypertonic medium, the incorporation of radioactive amino acids into protein decreases in both, but more severely in the uninfected cells. Although the effect of hypertonic medium on the synthesis of specific polypeptides varies, the translation of viral polypeptides as a class is less inhibited. The same patterns of proteins are synthesized regardless of the solute used in the hypertonic medium. The mechanism by which hypertonic conditions exert their effect on whole cells was investigated in K cell-free systems. It was possible to simulate the differential patterns of protein synthesis obtained in whole cells in hypertonic medium by increasing ion concentrations in cell-free extracts which are capable of initiating polypeptide chains on exogenous templates. However, in cell lysates which only elongate proteins, the same patterns were not obtained. Certain host and viral polypeptides displayed striking responses to increased ionic conditions in whole cells and cell-free systems. The synthesis of a host 44K protein, actin, appeared to be most sensitive; lower-molecular-weight proteins were fairly resistant. Among the viral proteins, the synthesis of 100K was inhibited, but most notable was the marked resistance of the synthesis of polypeptide IX. Possible mechanisms for differential synthesis and their significance are considered.

A great deal of attention is currently being focused on the primary structure of messenger RNA molecules and the relationship of specific sequences to the post-transcriptional control of gene expression. The preferental translation of certain messages under a given set of conditions, a process also known as differential translation, is one phenomenon responsible for the regulation of gene products. Examples of this type of regulation occur during infection of mammalian cells with a variety of viruses, such as poliovirus and adenovirus. The infected cell provides a natural environment in which the heterogeneous sets of viral and host messages coexist. At some point during infection, differential translation results in the predominant synthesis of viral proteins.

The mechanism of this switchover is still unclear, but it appears that viral messengers, as a class, have higher affinities for the translation machinery than do the host messages. This can be demonstrated by subjecting infected cells to hypertonic growth medium, thereby reducing the overall rate of initiation of protein synthesis (hypertonic initiation block [HIB]). With both RNA and DNA viruses, the synthesis of viral proteins is relatively resistant to the treatment, whereas host protein synthesis is severely inhibited, regardless of the solute used to increase the medium tonicity (15, 16, 23).

Although viral transcripts are generally more resistant to hypertonic conditions than are host messages, differences in the relative translational capacities of particular host and viral messages have been demonstrated (13-17).

The hypothesis proposed by Lodish (11) may provide an explanation for differential protein synthesis. Control of translation is assumed to be at the level of initiation (5, 10). Lodish suggests that any limitation in the reactants of the initiation process or any nonspecific decrease in the rate of initiation will reveal those messages which, for some reason, have a stronger affinity for the available protein synthetic components. The result would appear as a relative increase in the synthesis of proteins encoded by such messages. It was of interest to us to determine which proteins would be most affected under the limiting conditions created by increased ionic concentrations and to establish the role of HIB in limiting initiation.

In this communication we report data which indicate that the observed differential translation in both normal KB cells and adenovirus

type 5 (Ad5)-infected KB cells in hypertonic media may be accounted for by small increases in the intracellular concentration of ions, probably monovalent cations, as suggested by Carrasco (2) and Carrasco and Smith (3) for picornavirus infection. Ionic changes would most likely result from alterations in the cell membrane (2, 9). We show that in a KB cell-free system which is capable of initiating protein synthesis on exogenous messages, increased KCl alters the patterns of polypeptides synthesised in response to total KB cytoplasmic RNA and to total cytoplasmic RNA extracted from KB cells late after adenovirus infection. The altered patterns closely resemble those derived from normal KB cells and adenovirus-infected KB cells which have been exposed to hypertonic media.

(These results were taken from a Ph.D. thesis submitted by Claudia S. Cherney to the School of Medicine and Dentistry, University of Rochester, Rochester, N.Y.)

MATERIALS AND METHODS

Cell cultures and virus. Procedures for the maintenance of the KB human cell line and the propagation of Ad5 have been described previously (1). Cells collected from suspension cultures were washed twice with cold phosphate-buffered saline (10 mM sodium phosphate, pH 7.2, 2.7 mM KCl, 137 mM NaCl). Cells were infected 18 to 20 h before collection or labeling; 150 to 200 PFU of Ad5 per cell was used in infections for cytoplasmic extracts and extraction of RNA, and 10 PFU/cell was used to infect monolayer cells for pulse-labeling.

Pulse-labeling of cells in hypertonic media. Hypertonic media were made by adding NaCl or sucrose to Eagle minimal essential medium without serum. Normal or infected cells in Linbro plates (400,000 cells per experimental sample) were washed with isotonic Eagle minimal essential medium and incubated in the appropriate hypertonic medium for 20 min at 37°C.

For the determination of incorporation of labeled amino acids into total cell protein, the incubation medium was replaced with a pulse medium of the same tonicity, which contained 2.0 μCi of [^{35}S]methionine per ml (more than 700 Ci/mmol; Amersham/Searle), and cells were incubated for 30 min at 37°C. The medium was removed, and the cells were washed twice with cold phosphate-buffered saline, after which 0.2 ml of 10 mM Tris-chloride, pH 8.0, containing 0.01% sodium dodecyl sulfate (SDS) was added. After 5 min on ice, the lysate was precipitated in 10% trichloroacetic acid containing 0.1% Casamino Acids and 0.01% methionine. Precipitates were boiled for 10 min, cooled, and filtered onto nitrocellulose filters which were washed with 5% trichloroacetic acid and 80% ethanol. Incorporated radioactivity was determined by liquid scintillation counting of the filters.

To analyze the polypeptide products, the labeling procedure was the same as that described above, except that radioactive label in the pulse medium was increased to 5 μCi/ml for isotonic medium and further adjusted to compensate for the decreased incorporation which occurred in media of increased tonicity. After the labeling period and the Tris–SDS treatment, portions were precipitated with trichloroacetic acid to determine incorporation. The remaining lysates were prepared directly for gel analysis.

Preparation of cell-free systems. Cytoplasmic extracts from normal and infected cells used for endogenous synthesis experiments were prepared as described previously (30).

The preincubated extract from normal cells was prepared essentially by the method of Villa-Komaroff et al. (25).

Preparation of total cytoplasmic RNA. Total cytoplasmic RNA was extracted from the 10,000-×-*g* supernatant of normal and infected KB cells after they were swollen in hypotonic medium and disrupted by Dounce homogenization. The 10,000 -×-*g* supernatant was brought to a final concentration of 100 mM NaCl, 5 mM EDTA, and 0.5% SDS. To isolate RNA, a standard alkaline extraction previously described (31) was modified as follows: isoamyl alcohol was omitted from the organic phase, and phenol and chloroform were added sequentially during extractions. Ethanol-precipitated RNA was washed twice with 70% ethanol–10 mM Tris-chloride, pH 7.6, dissolved in water, and stored at −20°C.

Amino acid incorporation assays in cell-free extracts. Reaction conditions have been described previously (25). In a final volume of 50 μl, reactions with preincubated extract contained the following: 20 μl of extract having an absorbance at 260 nm of 25 to 50 U per ml; 30 mM HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid), pH 7.6; 1 mM ATP; 0.2 mM GTP; 1.0 mM dithiothreitol; 10.4 mM creatine phosphate (dipotassium salt); 100 μg of creatine phosphokinase per ml; the appropriate 19 unlabeled amino acids, each at 125 μM; 5 μCi of [^{14}C]-leucine (270 mCi/mmol; New England Nuclear Corp.) per ml or 100 μCi of [^{35}S]methionine (770 Ci/mmol; Amersham/Searle) per ml; 3.0 mM magnesium acetate; 110 to 150 mM KCl; and 200 μg of KB or Ad5-infected KB cell total cytoplasmic RNA per ml. Incubations were for 40 min at 31.0°C. Reactions were stopped and precipitated by the addition of 10% trichloroacetic acid (containing 0.1% Casamino Acids for reactions with [^{14}C]leucine). Precipitates were incubated for 10 min at 100°C to hydrolyze peptidyl tRNA. Samples were filtered and counted for incorporated radioactivity as described above.

Assays using endogenous systems were carried out under the same conditions, except for the differences in final KCl concentrations indicated in the figures and the fact that reaction volumes were 40 μl.

Gel electrophoresis of polypeptides from monolayer and cell-free reactions. [^{35}S]methionine-labeled, trichloroacetic acid-precipitated products were prepared for slab gel electrophoresis as described previously (30). The relative intensities of protein bands from autoradiograms were determined by scanning densitometry with a Beckman Computing CS-100 scanning densitometer. These results were confirmed by planimetry of the tracings.

RESULTS

Effect of hypertonic media on protein synthesis in KB cells and Ad5-infected KB cells. To determine the overall rate of protein synthesis during exposure to hypertonic media, uninfected cells and cells at 18 h (late) after virus infection were incubated in NaCl or sucrose hypertonic Eagle minimal essential medium containing [^{35}S]methionine. The amount of radioactive label incorporated into protein at various hypertonicities was measured. The overall rate of protein synthesis clearly declined (Fig. 1). Although the decline was more marked in media with added sucrose, a consistent observation with both NaCl and sucrose was that total protein synthesis in infected cells was less sensitive to hypertonic conditions than it was in normal KB cells. For example, with 100 mM excess NaCl, methionine incorporation by uninfected cells was less than 40% of the incorporation obtained in isotonic conditions; in contrast, incorporation by infected cells was 80% of the isotonic level.

To determine whether hypertonic conditions specifically affected the synthesis of particular proteins, normal and infected cells were labeled with [^{35}S]methionine in a series of media containing increasing additions of sucrose or NaCl. The proteins were solubilized and subjected to SDS-polyacrylamide gel electrophoresis, and the gels were autoradiographed (Fig. 2 and 3). The amount of [^{35}S]methionine used to label cells was increased to compensate for the decrease in protein synthesis which occurred in the most hypertonic media. Also, the final samples applied to the gels from each comparative experiment contained equal amounts of radioactivity (except where noted in the figure legends).

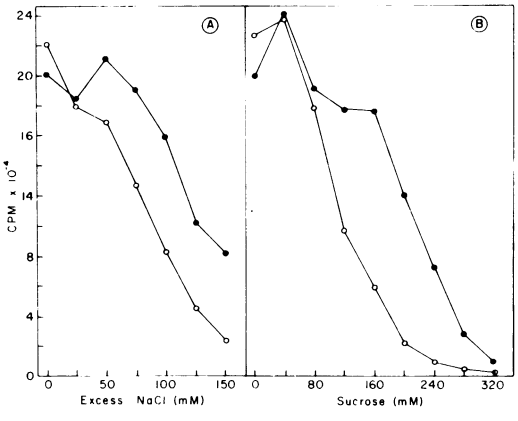

FIG. 1. *Effect of hypertonic media on total protein synthesis in normal and adenovirus-infected KB cells. Determination of [^{35}S]methionine incorporated into protein is described in the text. (A) Cells were pulse-labeled in media containing excess NaCl. The basal concentration of NaCl was 116mM (zero excess). At 150 mM excess NaCl the medium osmolarity was increased by 300 mosM. (B) Labeling of cells was carried out in media containing 120 mM NaCl and additions of sucrose. Sucrose at 320 mM increased the medium osmolarity by 320 mosM. Symbols ○, total radioactivity incorporated by uninfected cells: ●, incorporation by infected cells.*

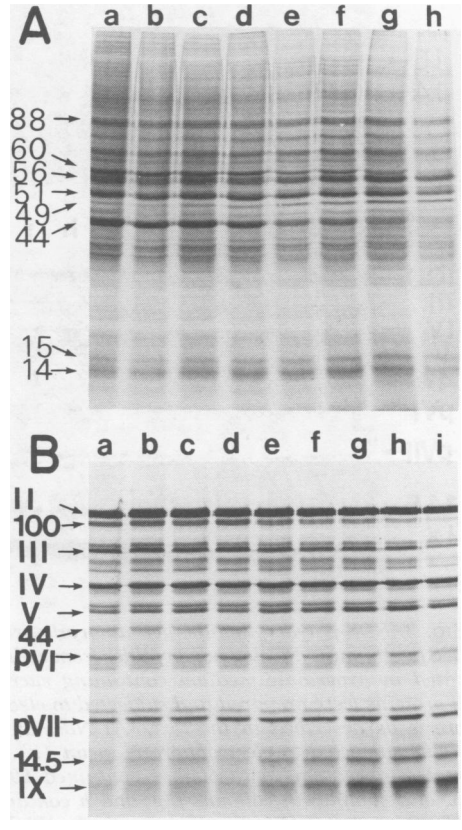

FIG. 2. *SDS-polyacrylamide gel autoradiograms of [^{35}S]methionine-labeled polypeptides from cells incubated in hypertonic medium containing excess NaCl. Cell proteins were prepared and subjected to electrophoresis as described in the text. (A) Normal KB proteins subjected to electrophoresis in an 8% gel. Columns a through g received 20,000 cpm, and column h received 10,000 cpm. (B) Proteins from infected cells subjected to electrophoresis in 8.7% gels. All column samples contained 30,000 cpm. Column a, medium contained basal NaCl (120 mM); column b, 20 mM excess NaCl; column c, 40 mM excess NaCl; column d, 60 mM excess NaCl; column e, 80 mM excess NaCl; column f, 100 mM excess NaCl; column g, 120 mM excess NaCl; column h, 140 mM excess NaCl; column i, 160 mM excess NaCl. The numbers used to label polypeptide bands refer to molecular weights ($\times 10^{-3}$) as estimated by their electrophoretic mobility relative to standards. Roman numeral designations of viral polypeptides are explained in the text.*

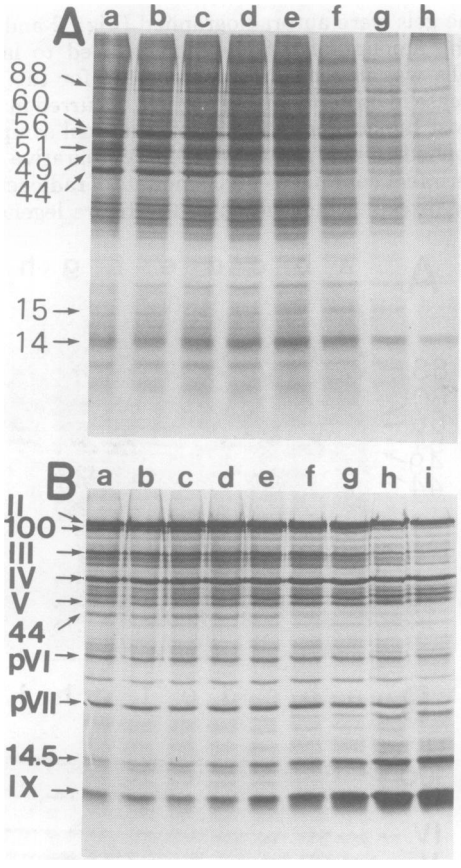

FIG. 3. *SDS-polyacrylamide gel autoradiograms of [^{35}S]methionine-labeled polypeptides from cells incubated in hypertonic medium containing sucrose. Cell proteins were prepared and subjected to electrophoresis as described in the text. (A) Normal KB proteins subjected to electrophoresis in an 11% gel. Samples in columns a through e contained 60,000 cpm, and samples in columns f, g, and h contained 40,000, 20,000, and 15,000 cpm, respectively. (B) Proteins from infected KB cells subjected to electrophoresis in an 11% gel. Columns a through g received samples containing 60,000 cpm; columns h and i, received 20,000 cpm (for this photograph, these columns were exposed three times longer). Column a, media contained 120 mM NaCl and no sucrose; column b, 40 mM sucrose added; column c, 80 mM sucrose added; column d, 120 mM sucrose added; column e, 160 mM sucrose added; column f, 200 mM sucrose added; column g, 240 mM sucrose added; column h, 280mM sucrose added; and column i, 320 mM sucrose added. Bands are designated as described in the legend to Fig. 2.*

Therefore, the autoradiogram patterns show changes in the synthesis of polypeptides relative to one another.

In both uninfected and infected cell patterns, progressive changes were clearly apparent as the medium tonicity was increased. To quantitate the relative changes (Fig. 4 and 5), densitometer tracings were made from each autoradiogram column. The most intense band in a column registered as the highest peak in the scan, and all other bands had proportional heights. The total area of the tracing and the areas of specific peaks were determined by planimetry for each consecutive column of the autoradiogram (Fig. 2 and 3). The area under a peak was expressed as a percentage of the total area and was taken as the percentage of the total labeled protein. Two lines of evidence suggest that the method gave a faithful representation of the patterns of labeled proteins. First, from a given sample various amounts of total radioactivity could be applied to the gel without significantly changing the relative areas of the peaks (data not shown). And second, a few autoradiogram patterns were randomly chosen, their corresponding dried gels were fractionated, and the distribution of label was determined by scintillation counting. The results agreed well with the distributions determined by planimetry (data not shown).

From each of the normal cell patterns (Fig. 2A and 3A), three clearly resolved bands were chosen to demonstrate the relative changes observed in response to hypertonic media. We have

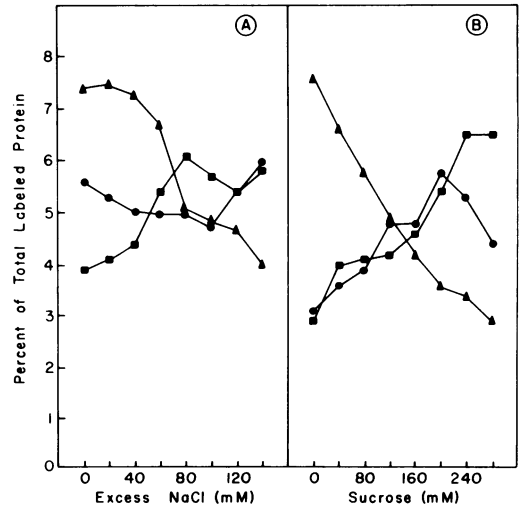

FIG. 4. *Relative synthesis of specific KB cell polypeptides in hypertonic media. The amount of a particular protein synthesized for each hypertonic concentration is expressed as a percentage of the total protein, calculated as described in the text. (A) Cells labeled in media containing excess NaCl, as in Fig. 2A (B) Cells labeled in media containing sucrose, as in Fig. 3A. Estimated molecular weights of representative host proteins are 88,000 (●), 44,000 (▲), and 14,000 (■).*

designated these 88K, 44K, and 14K; they have electrophoretic mobilities corresponding to 88,000 to 90,000, 44,000, and 14,000 daltons. The 88K protein showed only a slight increase in cells exposed to a hypertonic medium. In contrast, the 14K protein, whose abundance and mobility suggest that it may be a histone, increased notably from less than 4% to more than 6%. However, the most marked response was observed in the 44K protein (probably actin); it declined from more than 7 to 4% or less of the total protein (Fig. 2A, columns a through h; 3A, columns a through h; and 4). These changes may seem small, but one must view them in the light of the vast number of cellular proteins which are synthesized simultaneously. Other proteins, some of which were less well resolved, also exhibited graded responses to progressive HIB. Of those proteins designated in the figures, the visibly apparent changes were the decline in the 60K protein and the rise in the 56K and 49K proteins.

The influence of hypertonic media on the pattern of infected cell protein synthesis is shown in Fig. 2B and 3B. These autoradiograms from 18 h postinfection illustrate the outstanding viral polypeptide pattern obtained at late times after virus infection, when host protein synthesis is depressed. Because of the diminished host background, changes in the viral pattern are very dramatic. Certain viral proteins, such as 100K and III (penton base) proteins, were less efficiently synthesized in cells exposed to hypertonic medium; the syntheses of proteins IV (fiber) and pVII were maintained in a relatively constant proportion. In contrast, the 14.5K protein and polypeptide IX showed relative increases. The change in polypeptide IX was quite striking; this polypeptide represented 5% of the total labeled protein obtained in isotonic medium, but its incidence rose to account for as much as 35% of the total protein synthesized by cells exposed to the most extreme hypertonic conditions (Fig. 2B, columns a through i; 3B columns a through i; and 5).

Although the most readily demonstrable responses to hypertonic media occurred in viral proteins, host cell proteins also displayed differential sensitivities to HIB, as we have shown here and as reported by others. The similarity of the qualitative responses of normal and infected cells supports the notion that the translation of viral RNAs is subject to the same general mechanisms of regulation as is the translation of host messengers. Some possible modes of regulation are discussed below.

Influence of increased KCl concentration on protein synthesis in cell-free systems derived from normal and infected cells. We have observed by microscopy that cells bathed in hypertonic medium shrink, as noted by others (see below and reference 28). Therefore, we assume that a primary physiological effect of hypertonic media on cultured cells is a decrease in intracellular fluid, which results in an increase in intracellular ion concentrations. To investigate the effect(s) this may have on protein synthesis, experiments were carried out in KB cell-free systems in which the ionic conditions could be directly manipulated and the resulting changes in protein synthesis could be assessed. Two types of cell-free systems were used: one, prepared from either normal or infected cells, was a post-mitochondrial lysate, in which polysomes elongate proteins on endogenous messages, but fail to initiate new chains; the other system, made only from normal cells, was a preincubated lysate containing run off ribosomes which are active in initiation and elongation of

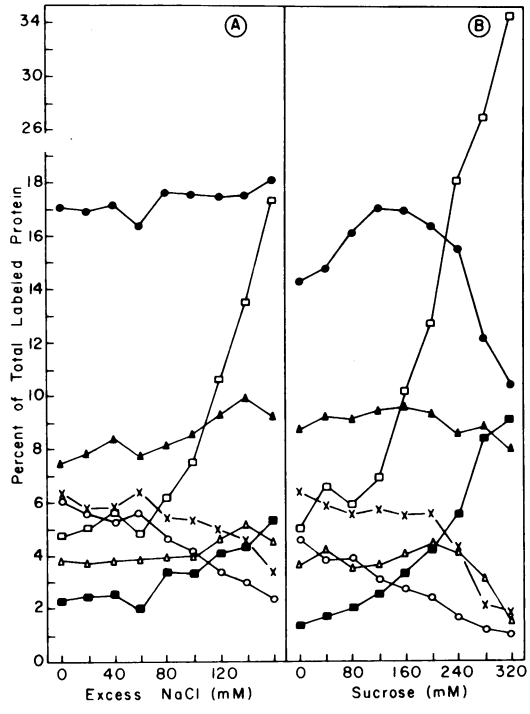

FIG. 5. *Relative synthesis of specific polypeptides from infected KB cells in hypertonic media. Conditions are described in Fig. 4. (A) Cells labeled in media containing excess NaCl, as in Fig. 2B. (B) Cells labeled in media containing sucrose, as in Fig. 3B. Symbols: ●, protein II; ○, protein 100K; ×, protein III; ▲, protein IV; △, protein pVII; ■, protein 14.5K; and □, polypeptide IX.*

proteins. The advantage of the preincubated system is that it is stimulated by the addition of exogenous messenger; protein synthesis can be directed by total cytoplasmic RNA extracted from normal cells (KB RNA) or from KB cells 18 h after adenovirus infection (Ad5-KB RNA). To substantiate the initiating capacity of the preincubated extract versus the endogenous extract, heparin, an inhibitor of initiation (26), was added to both types of cell-free system. Only the preincubated system with added mRNA was significantly inhibited (Table 1).

When the KCl concentration of the preincubated system was varied from 100 to 150 mM or more, the incorporation of radioactive amino acids into protein declined. Data for leucine and methionine are shown in Fig. 6; the decline was less notable with methionine, probably because the background incorporation without added RNA was higher. Nevertheless, in both cases the decrease was most marked when KB RNA was added to the system. Figures 6B and D show the amount of protein synthesized at a particular KCl concentration relative to the maximum amount synthesized after subtraction of incorporation occuring in the absence of added RNA. The results indicate that maximal synthesis occurred at 130 mM KCl for KB RNA and Ad5-KB RNA. With only a 10 mM increase in KCl, the synthesis directed by KB RNA fell to 40% of the maximum, whereas that directed by Ad5-KB RNA decreased much less, to about 75% of the maximal level. It should be noted that these effects correlate well with the sensitivities of intact normal and infected cell protein synthesis to HIB. In our hands, the KCl concentration which was optimal for cell-free protein synthesis varied slightly with each extract, but a 10 mM increase beyond the optimum consistently inhibited the translation of KB RNA more than that of Ad5-KB RNA. The effect could still be seen with extreme inhibitions; in the leucine experiment, KCl at 160 mM resulted in only 10% of maximal synthesis in the KB RNA-driven system, but it resulted in 20% of maximal synthesis in the Ad5-KB RNA-driven system. In contrast, when the KCl concentration was increased in the endogenous, non-initiating system, only a small decline in synthesis occurred. At 160 mM KCl, incorporation of [^{14}C]leucine in the KB RNA system was at least 60% of the maximal level, and it was 80% in the Ad5-KB RNA system (data not shown).

The differential polypeptide responses elicited by increased KCl concentration in the preincubated system were quite similar to those observed in intact cells exposed to HIB. The autoradiograms of the [^{35}S]methionine-labeled polypeptides synthesized in the preincubated system clearly demonstrate that those cellular proteins which were strongly affected by HIB appear to be the same proteins which were influenced by increased KCl in the messenger-dependent, initiating cell-free system. In the KB RNA pattern (Fig. 7A), the most notable change was the decrease in the 44K protein, and in the Ad5-KB RNA product pattern (Fig. 7B), the increase in polypeptide IX was quite marked. However, these changes were not observed when the endogenous, elongating, cell-free systems were subjected to increased KCl concentrations (Fig. 8). A comparison of the effect of increased KCl on relative amounts of particular proteins (as explained for Fig. 4 and 5) from the preincubated and endogenous systems (Fig. 9) shows that other minor changes which were similar in the preincubated system and in intact cells (such as the relative decline in viral polypeptide III) were also not observed in the endogenous system.

These results strongly suggest that differential translation caused by increased KCl was pri-

TABLE 1. *Incorporation of [^{14}C]leucine in KB cell-free systems in the presence of the initiation inhibitor heparin*

System[a]	Incorporation into control (cpm)	Incorporation (cpm) with the following concn of heparin:		
		25 μg/ml	50 μg/ml	100 μg/ml
KB endogenous	6,500 (100)[b]	7,500 (115)	6,200 (96)	ND[c]
Ad5-infected KB endogenous	26,700 (100)	28,800 (108)	25,900 (97)	24,200 (91)
KB (preincubated) + KB RNA[d]	1,500 (100)	360 (24)	30 (2)	200 (13)
KB (preincubated) + Ad5-KB RNA[d]	3,300 (100)	320 (10)	420 (13)	220 (7)

[a] Cell-free systems were prepared and assayed as described in the text.

[b] Counts per minute of total trichloroacetic acid-precipitated protein. Numbers in parentheses are percentages of control values.

[c] ND, Not determined.

[d] For each determination, the counts per minute observed in reactions without added RNA has been subtracted.

marily dependent upon changes in the rate of initiation of specific messages. The correlation with peptide patterns from intact cells exposed to HIB supports this. Furthermore, when overall initiation in cell-free systems was limited by heparin or high temperature, only polypeptide IX and a few other HIB-resistant proteins were synthesized (manuscript in preparation). It is likely that these proteins are synthesized from the messages which participate most efficiently in the initiation process.

DISCUSSION

The results presented here are in agreement with recent reports of the inhibition of host and viral translation by hypertonic conditions. We have found that the inhibition of protein synthesis in KB cells exposed to hypertonic media was less pronounced in cells previously infected by adenovirus. Also, when the KCl concentration was increased in a preincubated KB cell-free system, the translation of viral messages was less inhibited than was the translation of host messages. The inhibitory effect appears to be at the level of initiation, based upon comparisons of the protein synthesis patterns of initiating and non-initiating cell-free systems. In the cell-free system which sustains initiation of polypeptide chains, the synthesis patterns obtained with increasing KCl concentrations very closely resemble the patterns from whole cells in hypertonic media for both host and viral products. Synthesis patterns did not change in systems which allow only elongation of pre-initiated chains.

Therefore, we suggest that cells exposed to hypertonic medium experience increased internal ionic concentrations which affect translation primarily at the level of initiation. It is known that potassium can also affect the rate of elongation in vitro (12) and therefore can alleviate some deficiencies in the synthesis of high-molecular-weight proteins in cell-free systems. The synthesis of the larger adenovirus proteins is enhanced in the Krebs ascites system by the addition of KCl (29). If the range of KCl concentrations used in our studies were affecting elongation in our preincubated system, we would expect to have seen a relative increase in the high-molecular-weight products. No such increase was observed. Indeed, polypeptide II declined slightly and 100K protein substantially declined (Fig. 7B). Although increased KCl did produce changes in the total amount of endogenous protein synthesized (data not shown), there were no significant changes in the polypeptide patterns (Fig. 8). We conclude, therefore, that although increased KCl may have a uniform overall effect on the rate of elongation in our cell-free systems, it only produces a differential effect on the rate of initiation.

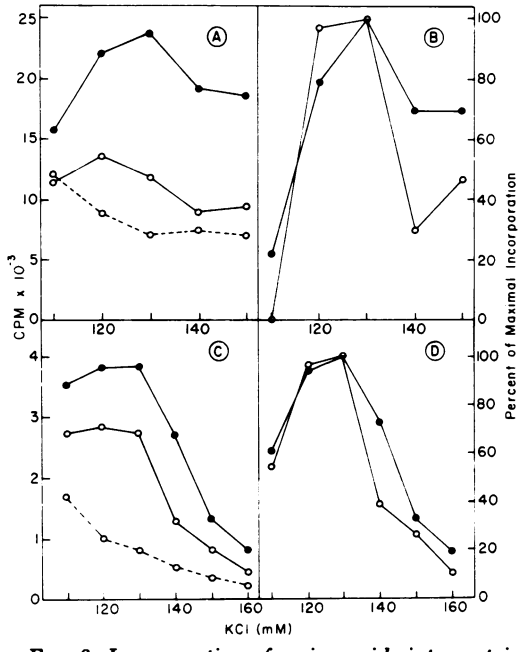

FIG. 6. *Incorporation of amino acids into protein at different KCl concentrations in the preincubated KB cell-free system. Protein synthesis assays, as described in the text, contained no added RNA (O--O, background), KB RNA (O—O), or Ad5-KB RNA (●—●). (A) Incorporation of [^{35}S]methionine; (C) incorporation of [^{14}C]leucine. (B) and (D) Incorporation of [^{35}S]methionine and [^{14}C]leucine, respectively, expressed as a percentage of the total counts incorporated at the KCl concentration which allowed maximum synthesis. Incorporated radioactivity in background reactions was subtracted for each determination before calculation of percentages.*

The most striking effect of hypertonic media on host protein synthesis was the decrease in a very abundant 44K polypeptide, which we assume to be actin, based on its amount and electrophoretic mobility. The sensitivity of this protein in BSC-1 cells exposed to HIB has been reported by others (17). That actin seemed to be one of the few host proteins significantly synthesized late in infection (after the switch to viral synthesis had occurred) was somewhat unexpected. This phenomenon may be a manifestation of the abundance of actin mRNA. However, actin synthesis was still more sensitive to HIB than was viral protein synthesis.

We also point out three host proteins which are relatively resistant to HIB and which appear to increase: a 49K protein and two low-molecular-weight proteins which have electrophoretic mobilities similar to those of histones. These

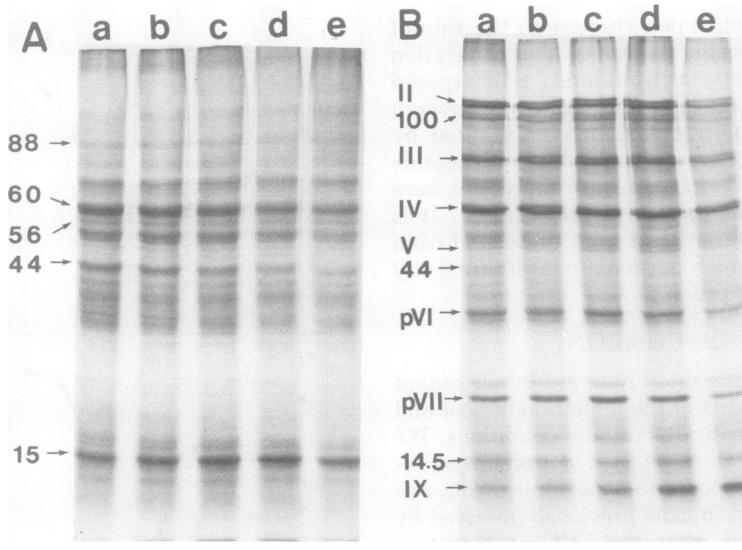

FIG. 7. *SDS-polyacrylamide gel autoradiograms of [^{35}S]methionine-labeled products of the KB preincubated cell-free system at different KCl concentrations. Protein synthesis assay conditions are described in the text. (A) Products from reactions with added KB RNA (B) Products from reactions with added Ad5-KB RNA. Each sample contained 40,000 cpm. Column a, 110 mM KCl; column b, 120 mM KCl; column c, 130 mM KCl; column d, 140 mM KCl; and column e, 150 mM KCl. Bands are designated as described in the legend to Fig. 2.*

three proteins may correspond to the resistant species of 50,000, 13,000, and 12,000 daltons reported by Opperman and Koch (17).

A remarkable change was observed in the protein synthesis pattern of infected cells exposed to HIB. The small polypeptide IX increased to an exceptionally high proportion of the total labeled protein: nearly one-third at 320 mM sucrose. Because this result seemed so unusual and because it was derived from a densitometry scan of a somewhat diffuse band, we entertained the possibility that cells in hypertonic media may experience increased processing of higher-molecular-weight proteins to smaller products. This might also be responsible for the observed slight increases in labeling intensity of a number of lower-molecular-weight bands. To test this hypothesis, normal and infected cells were pulse-labeled in isotonic media and chased in either isotonic or hypertonic media, and the labeled proteins were analyzed on gels. Patterns of cells chased in isotonic and hypertonic media were identical (data not shown), suggesting that the increase in label in the polypeptide IX band cannot be explained by comigration of cleavage products.

Under conditions which limit the overall rate of initiation of protein synthesis, one would expect those proteins whose messages are most efficient to account for an increased proportion of the total protein. Under normal conditions,

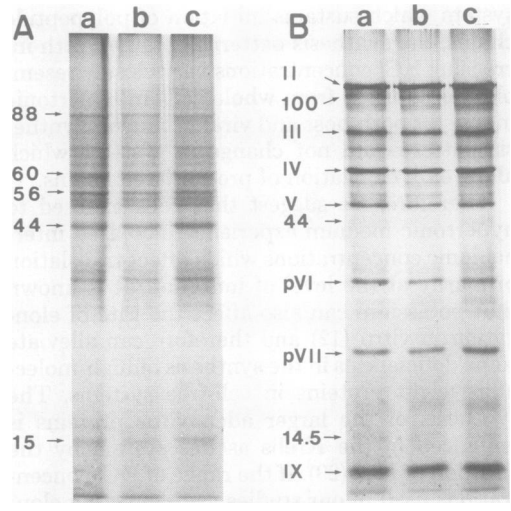

FIG. 8. *SDS-polyacrylamide gel autoradiograms of [^{35}S]methionine-labeled polypeptides of endogenous KB cell-free systems at different KCl concentrations. Protein synthesis assay conditions are described in the text. (A) Products of endogenous KB system. (B) Products of endogenous system from adenovirus-infected KB cells. Each sample contained 150,000 cpm. Column a, 120 mM KCl; column b, 140 mM KCl; column c, 160 mM KCl.*

the apparent proportion of a protein is partly dependent on the abundance of its mRNA. At present, there are no quantitative data available

on the relative abundance of the mRNA for polypeptide IX at late times after infections. Recent data (6) suggest that for Ad2 the most abundant messages are from the right end of the genome, the region coding for the 100K protein and the large structural proteins (for example, proteins II and IV). When the amounts of proteins synthesized at late times after Ad5 infection in KB cells under isotonic conditions were compared on a molar basis, we found 2.5 times more polypeptide IX than protein II and more than 3 times more polypeptide IX than protein IV (and the relative synthesis of polypeptide IX appeared to be even higher in cell-free products). This suggests that polypeptide IX arises from an abundant messenger and agrees with the data of Persson et al. (19).

However, when initiation is limited, the relative proportion of polypeptide IX exceeds its normal value, indicating that the differential response of polypeptide IX to HIB is related to factors other than messenger abundance. We favor the idea that the structure of the mRNA molecule is critical for translation efficiency. All late Ad2 messages, with the notable exceptions of protein IVa and polypeptide IX, have common spliced, noncoding sequences of about 150 nucleotides at the 5′ end (4, 8). Polypeptide IX and protein IVa messengers have 5′ spliced sequences, but they are different from the common leader. Furthermore, the size of the message for polypeptide IX is 550 nucleotides (21), of which 330 nucleotides would be needed for coding and 150 to 200 nucleotides may be polyadenylic acid, allowing for a noncoding leader sequence which is much smaller than the common leader of the other late messages. The small distance between the coding region for polypeptide IX and the cap may facilitate the protection of the cap by the ribosome and allow for a stronger binding. There is evidence that the presence of an intact cap becomes more critical for efficient translation with increased ionic conditions (27). The interaction of the cap with the ribosome could well play a role in resistance to HIB and, indeed, may account for the differential resistance of proteins of vesicular stomatitis virus (13, 22). It is also possible that particular messenger sequences contribute to HIB resistance by interacting with initiation factors or by pairing with complementary rRNA sequences, as has been shown in procaryotes (24) and suggested for eucaryotes (7). Recently, it has been suggested that polypeptide IX may represent a "quasi-late" viral product, a type previously unrecognized in adenovirus infections (20). This property and the unusual resistance of polypeptide IX to HIB indicate that polypeptide IX may have a role in infection other than its structural function in the virion.

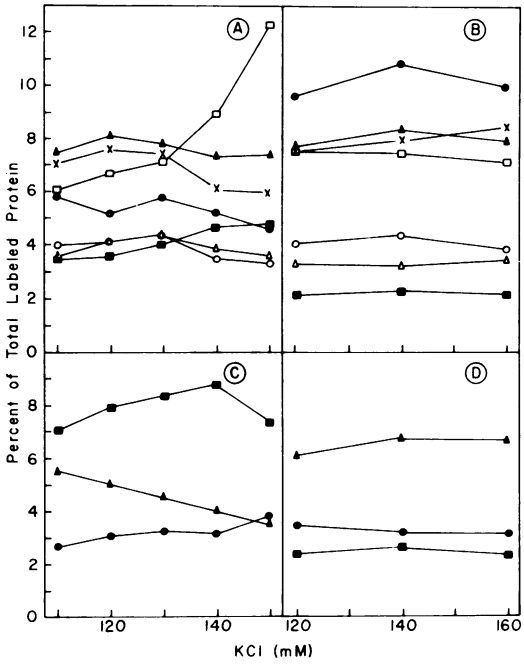

FIG. 9. *Relative synthesis of specific polypeptides from KB cell-free systems at different KCl concentrations. The results are derived from the autoradiograms in Fig. 7 and 8 and are expressed as percentages of the total labeled protein, as described for Fig. 4 in the text. (A) Preincubated KB cell-free system with Ad5-KB RNA added (from Fig. 7B). (B) Endogenous system of infected cells (from Fig. 8B). Symbols: ●, protein II; ○, protein 100K; ×, protein III; ▲, protein IV; ■, protein 14.5K; □, polypeptide IX. (C) Preincubated KB cell-free system with KB RNA added (from Fig. 7A). (D) Endogenous system of KB cells (from Fig. 8A). Symbols: ●, protein 88K; ▲, protein 44K; ■, protein 15K.*

Whether the observed responses to altered ionic conditions have any actual physiological significance remains to be determined. However, a shift in intracellular ion concentration during the infectious cycle could conceivably cause a general limitation on initiation, allowing viral synthesis a relative translational advantage and facilitating host shutoff. Based on evidence from picornavirus studies, Carrasco (2) proposed a mechanism in which ion(s) specifically inhibit host translation while stimulating viral translation. The fact that polyribosomes are smaller in adenovirus-infected cells than in uninfected cells (18) is consistent with a depression in the overall rate of peptide chain initiation. At present we have preliminary data which indicate that cell-associated Na^+ and K^+ do, indeed, increase throughout adenovirus infection of cultured cells.

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