Control of Peptide Chain Initiation in Uninfected and Virus Infected Cells by Membrane Mediated Events

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Abbreviations

MEM	Minimal Essential Medium
HIB	Hypertonic Initiation Block
VSV	Vesicular Stomatitis Virus
TPCK	L-1-Tosylamido-2-Phenylethyl Chloromethyl Ketone
DMSO	Dimethylsulfoxide
L	Immunoglobulin Light Chain
H	Immunoglobulin Heavy Chain
DEAE	Diethylaminoethyl-dextran
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

Summary

Initiation of protein synthesis in tissue culture cells is rapidly inhibited or blocked by addition of either DMSO, ethanol, TPCK, cytochalasin B, or sucrose to the growth medium. In contrast, these agents do not interfere with the initiation of protein synthesis in cell-free extracts to a comparable extent. These results support the hypothesis that protein synthesis in tissue culture cells can be influenced by membrane mediated events.

Translation of viral mRNA in RNA virus infected cells is resistant to a number of these inhibitors of peptide chain initiation and proceeds under conditions where translation of host mRNA is almost completely suppressed. It appears that viral mRNA possesses a greater ability than host mRNA to form mRNA-ribosome initiation complexes when the overall rate of peptide chain initiation is reduced. This observation has led to a number of predictions concerning the strategy of virus directed suppression of host mRNA translation.

Under optimal growth conditions protein synthesis appears to be regulated mainly, but not exclusively, by the amount of the mRNA available for translation. However, when cellular growth and/or the overall rate of peptide chain initiation is restricted, control of protein synthesis at the translational level becomes decisive with the translation of each mRNA species proceeding with its own characteristic efficiency, most probably as a result of inherent differential affinities of individual mRNA species for ribosomes.

Introduction

In spite of extensive studies, much remains to be determinded concerning mechanisms of translational control in tissue culture cells. We have previously suggested that this control can be exerted by membrane mediated events (Pong, Nuss and Koch, 1975).

An increase in the tonicity of the growth medium exerted by addition of either salt or sucrose (Oppermann, Saborio, Zarucki, and Koch, 1973) results in an immediate block in the initiation of protein synthesis (Saborio, Pong, and Koch, 1974), while elongation and termination of protein synthesis as well as processing, transport and secretion of proteins continue unabated. It is well known that infection of various tissue culture cell lines with a number of RNA or DNA viruses also results in an inhibition of cellular protein synthesis. This inhibition may result from competition between virus and host mRNAs for ribosome binding sites (Lawrence and Thach, 1974; Nuss, Oppermann and Koch, 1975) and/or a selective interference with host peptide chain initiation, perhaps due to the action of viral protein(s) (Matthews *et la.*, 1973; Wright and Cooper, 1974; Racevskis, Kerwar and Koch, 1976). However, the identification of a factor which can selectively suppress the translation of host mRNA has not been forthcoming. Interestingly, a brief exposure of virus infected cells to hypertonic medium does result in a striking preferential inhibition of host mRNA translation (Nuss, Oppermann and Koch, 1975). This result suggests that the efficiency with which translation can be initiated must be greater for viral mRNA than for host mRNA. That is, viral mRNA might possess a stronger affinity for ribosomes than host mRNA, resulting in only a slight reduction in its translation when the overall rate of peptide chain initiation is reduced, and thus gaining a translation advantage. This interpretation could readily explain why infection of cells by isolated viral mRNA is promoted under experimental conditions that interfere with cellular protein synthesis (Koch, 1973). The observations that a brief exposure of virus infected cells to hypertonic conditions early in the infectious cycle results in a potentiation of virus directed suppression of host translation leads to the proposal that the latter process can be partially explained by an early indiscriminate reduction in the overall rate of peptide chain initiation. Indeed, this event, coupled with competition between host and viral mRNA for ribosome binding sites later in the infectious cycle when viral mRNA becomes more abundant, may adequately explain suppression of host translation directed by a number of RNA containing viruses (Nuss, Oppermann and Koch, 1975).

Initiation of protein synthesis in HeLa cells *in vivo* is inhibited by a number of agents in addition to medium hypertonicity (Saborio, Pong and Koch, 1974), including DMSO (Saborio and Koch, 1973); ethanol (Koch and Koch, 1974); TPCK (Pong, Nuss and Koch, 1975); and cytochalasin B (Koch and Oppermann, 1975). We have studied the effect of several of these experimental conditions on protein synthesis in uninfected and virus infected cells and on *in vitro* protein synthesis in cell-free extracts prepared from mammalian cells. The results support the proposal that under a number of natural and experimental conditions the rate of peptide chain initiation can be regulated by membrane mediated events.

Materials and Methods

Cells and Viruses

Cells were grown in suspension as described previously (Saborio, Pong and Koch, 1974; Nuss and Koch, 1976a) or in monolayers (Oppermann and Koch, 1976a). Infection of cells by poliovirus, Type 1, strain Mahoney or by VSV, serotype Indiana (Mudd and Summers, 1970) was performed as described (Nuss, Oppermann and Koch, 1975).

Experimental Conditions for Isotope Labeling

Cells were transferred to serum free MEM (Joklik-modified, Gibco F13) + 25 mM Hepes, pH 7.4, containing no, or 1/20 the normal concentration of methionine. The tonicity of the medium was adjusted by addition of appropriate aliquots of a 4 M NaCl solution. Cells were incubated for 15 min at 37 °C (to allow run-off of ribosomes from those mRNA molecules on which initiation of translation has been blocked) (Saborio, Pong and Koch, 1974), followed by an additional 15 min in the presence of [^{35}S]methionine (New England Nuclear, above 250 Ci/mM). Aftter labeling, 50 µl aliquots were withdrawn and anlyzed according to Mans and Novelli (1961). The remainder of the cell suspensions was diluted 10 fold with ice cold chase-medium, containing 10 mM unlabeled methionine. The cells were centrifuged, resuspended in warm chase medium and incubated for another 15 min at 37 °C.

Lysis of Cells and SDS Polyacrylamide Gel Electrophoresis

Cytoplasmic extracts were prepared as previously described (Saborio, Pong and Koch, 1974; Nuss and Koch, 1976a), analyzed by polyacrylamide slab gel electrophoresis (Laemmli and Favre, 1973) and autoradiographed on medical X-ray film. Quantitation of [^{35}S]methionine incorporation into individual polypeptides was achieved in two ways; by measuring the area under the peaks on autoradiograph tracings or directly by excision and elution of individual peptide bands and determination of radioactivity by liquid scintillation spectrometry (Oppermann and Koch 1976 a).

Preparation of Cell-free extracts

Cell-free extracts were prepared from HeLa S_3 and mouse L cells according to the method of McDowell, *et. al.* (1972). The extracts were frozen in small aliquots in liquid N_3. An appropriate amount of extract was thawed immediately prior to use.

In vitro amino acid incorporation

The reaction mixtures (100 µl) contained 25 µl of cell-free extract, 1 mM ATP, 0.2 mM GTP, 220 µM creatine phosphate, 110 µg creatine phosphokinase, 25 mM HEPES-KOH, pH 7.6, 2.5 mM Mg acetate, 1 mM dithiotreitol, 2–10 µl [^{35}S]methionine (NEN), and 2–6 µg poly-A containing mRNA isolated from rabbit-peritoneal exudate cells (Koch, *et. al.*, 1976). Incubation was at 25 °C. The reaction was terminated by spotting aliquots on Whatman No. 3 paper discs (2.3 cm) prewetted with 50 µl of an amino acid mixture containing 5 mM L-methionine. The discs were processed as described by Mans and Novelli (1961).

Results

Inhibitors of protein synthesis: Effect on Protein Synthesis In Vivo and In vitro

Protein synthesis in tissue culture cells is rapidly inhibited or blocked by an increase in the tonicity of the growth medium (Saborio, Pong and Koch, 1974), DMSO (Saborio and Koch, 1973), ethanol (Koch and Koch, 1974), cytochalasin B (Koch and Oppermann, 1975), and by TPCK (Pong, Nuss and Koch, 1975). An increase in the osmolarity of the medium results in a selective inhibition of peptide chain initiation (Saborio, Pong and Koch, 1974). This inhibition is independent of the solute used to raise the osmolarity in the medium (Oppermann, et al. 1973) and is completely reversible upon return to isotonic condition. Addition of DMSO, cytochalasin B, or TPCK also preferentially effects peptide Chain initiation. However, DMSO also triggers a premature release of nascent peptides (Saborio and Koch, 1973) and cytochalasin B inhibits overall protein synthesis by only 50 % (Koch and Oppermann, 1975). TPCK, although as selective als hypertonic condition in its inhibition of peptide chain initiation, is not reversible *in vivo* (Pong, Nuss and Koch, 1975).

We have analyzed the effect of various *in vivo* inhibitors of peptide chain initiation on the translation of mRNA in cell-free extracts (Table 1). The data

Table I: Inhibition of Protein Synthesis in vivo and in cell-free Extracts

Addition	Conc.	Exposure (min)	amino acid incorporation % of control in vivo	amino acid incorporation % of control in vitro	Reversible in vivo	References
Sucrose	0.2 M	5–10	5	100	+	1
DMSO	12 %	3	2	50	+	2
Polycations (DEAE-dextran)	160 µg	30	10	0	(+)	3
Ethanol	2 %	1	60	100	+	4
Ethanol	3 %	1	10	70	+	4
Cytochalasin B	20 µg	30	50	100	(+)	5
TPCK	30 µg	5–10	2	80	–	6
Trypsin	500 µg	20	5	0	+	7
Pronase	100 µg	30	5	0	+	7
Glycopeptides	5–100 µg	5–10	0–80	0–80	+	8

Table 1: Protein synthesis *in vivo* in suspended HeLa S_3 or L cells was studied by following the incorporation of [^{35}S]methionine or [^{3}H]alanine into acid soluble proteins. The reversibility of the inhibition of protein synthesis *in vivo* was determined by sedimenting the cells and resuspension in fresh growth medium. Recovery of protein synthesis of better than 50 % (+) or 80 % + is listed in the second to last column in the table.

Protein synthesis *in vitro* was determined as described under Materials and Methods. The data on the inhibition of *in vivo* protein synthesis have been published in part previously (1. Oppermann, *et. al.*, 1973; 2. Saborio and Koch, 1973; 3. Saborio, Wiegers and Koch, 1975; 4. Koch and Koch, 1974; 5. Koch and Oppermann, 1974; 6. Pong, Nuss and Koch, 1975; 7. Koch, 1974; 8. Foch, Kubinski and Koch, 1974).

show that most of these agents inhibit protein synthesis to a much greater extent *in vivo* than *in vitro*. The different effects of these inhibitors on protein synthesis *in vivo* and *in vitro* have led to an inquiry as to whether the *in vivo* induced inhibition is a result of an indirect effect. That is, the primary action of these inhibitors might be exerted on the cell membrane, resulting in the activation and/or release of factor(s) which interfere with peptide chain initiation *in vivo*. This hypothesis is supported by the following observations: cell-free extracts, prepared from cells which had been exposed to either hypertonic conditions or TPCK *in vivo* for 15 min at 37 °C (the time required for complete run-off of ribosomes from polysomes) show no endogenous protein synthesis. Endogenous protein synthesis can be restored, however, by gel filtration over Sephadex G25 (Hoffman *et al.*, 1976) or by dialysis for 60 min at +4 °C, indicating that neither ribosomes nor mRNA are irreversibly inactivated by exposure of cells to TPCK or hypertonic conditions (McFarland and Koch, unpublished data).

Effect of inhibitors of peptide chain initiation and elongation on protein synthesis in various tissue culture cell lines and in virus infected cells

It was previously reported that one *in vivo* inhibitor of peptide chain initiation (excess NaCl) was less effective in inhibiting protein synthesis in poliovirus infected HeLa cells than in uninfected HeLa cells (Saborio, Pong and Koch, 1974; Nuss, Oppermann and Koch, 1975). This observstion has been extended and analyzed in detail in a number of uninfected and virus infected tissue culture cells (Nuss, Oppermann and Koch, 1975; Nuss and Koch, 1976a & b; Oppermann and Koch, 1976a & b). Although resistance of protein synthesis to hpertonic initiation block (HIB) varies from cell line to cell line and is higher when cells are grown in monolayer versus suspension (Oppermann and Koch, 1976a), a striking increase in resistance to HIB is observed following virus infection of cells (Fig. 1). Similar observations were obtained with other inhibitors of peptide chain initiation but not with inhibitors of peptide chain elongation such as puromycin and cycloheximide (Table 2). These results could be interpreted in several ways. Virus infection could result in changes in cell membrane structure, conferring a higher resistance of the synthesis of all proteins to HIB and other inhibitors of peptide chain initiation. Alternatively, viral mRNA translation selectively proceeds under conditions where host mRNA translation is severely inhibited resulting in the observed increased resistance.

Differential inhibition of mRNA translation by HIB

To further investigate the virus induced increase in resistance of protein synthesis to HIB, the synthesis of individual cellular proteins in uninfected cells and of host and viral proteins early in the replication cycle in several RNA and DNA virus infected cells was studied. Cells were pulse labeled under isotonic and hypertonic conditions and cytoplasmic extracts were subsequently analyzed by polyacrylamide gel electrophoresis (Nuss, Oppermann and Koch, 1975; Nuss and Koch, 1976a, b; Oppermann and Koch, 1976a, b). The results revealed that exposure of virus infected cells to appropriate hypertonic condition amplified the inhibition of host mRNA translation while viral mRNA translation was affected only slightly. We concluded therefore that viral mRNAs are more efficient messengers

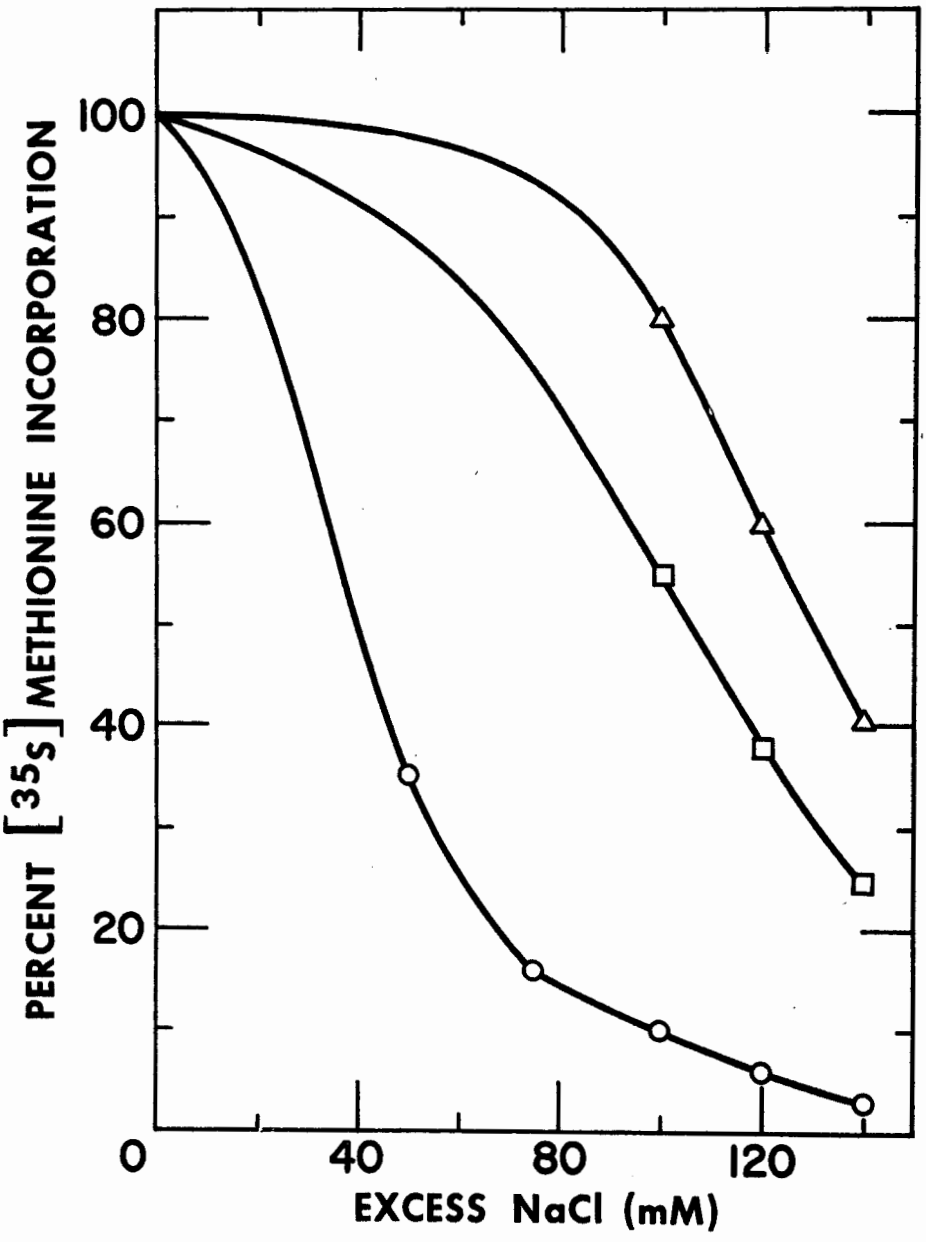

Fig. 1: Inhibition of protein synthesis in uninfected and virus infected HeLa cells by HIB. HeLa cells, uninfected (○—○), VSV infected (□—□) 300 min post infection or poliovirus infected (△—△) 180 min post infection were pulse labeled under isotonic or hypertonic conditions as described in the section on Materials and Methods.

than most host mRNAs and suggested that under conditions which result in a reduction in the overall rate of peptide chain initiation, each mRNA is translated with its own characteristic efficiency. To further investigate this hypothesis, the relative sensitivities of individual cellular mRNA translation to HIB were determined.

The mouse plasmacytoma cell line MPC-11 synthesizes an immunoglobulin gamma heavy (H) chain of approximately 55,000 daltons and a 23,000 molecular weight kappa light (L) chain (Laskov and Scharff, 1970). The L and H chains are reported to account for as much as 20 % of the newly synthesized polypeptides (Laskov and Scharff, 1970). Since the synthesis of the two IgG polypeptides make

Table II: Inhibition of Protein Synthesis in uninfected and Poliovirus infected HeLa cells

Condition	amino acid incorporation % of control	
	uninfected	infected.
Control	100	100
0.1 M NaCl excess	5	80
0.15 M NaCl excess	1	25
TPCK 30 μg/ml	2	30
50 μg/ml	1	20
ethanol 3 %	10	60
5 %	0	20
cytochalasin B 20 μg/ml	50	80
puromycin 10^{-3} M	10	10
cycloheximide 100 μg/ml	5	5

Table 2: HELa cells in suspension were infected with poliovirus or mockinfected and pulse labeled with [^{35}S]methionine 2.5 hrs post infection as described (Nuss, Oppermann and Koch, 1975) in the presence and absence of inhibitors of protein synthesis.

up a large proportion of the total protein synthesis and they can be easily identified and quantitated, MPC-11 cells provide a useful system to investigate the effect of HIB on the synthesis of several individual cellular polypeptides (Nuss and Koch, 1976a).

A comparison of the distribution of [^{35}S]methionine incorporation into MPC-11 polypeptides labeled under isotonic (panel A) and hypertonic (panel B) conditions is shown in Fig. 2. There is approximately a 3.5–4.0 fold increase in the relative incorporation into the L chain under hypertonic conditions (panel B) when compared to that observed under isotonic conditions (panel A). Likewise, there is an increase in the relative incorporation into the H chain polypeptide of approximately 1.5 fold. An estimation of the area under the curves for the L, H and total non-IgG proteins for this particular experiment reveals that the percent of total [^{35}S]methionine incorporation which is associated with the L chain, increases from a value of 6.9 % at isotonic conditions to a value of 27.2 % when cells are pulse labeled under hypertonic conditions. This value increases from 8.8 % to 12.8 % for the H protein. These results suggest that the mRNAs coding for the specialized IgG polypeptides are, as viral mRNA, efficient messengers relative to mRNA coding for other cellular proteins.

The experiment described in Fig. 2 was repeated with a number of other cell lines to compare the translational efficiencies of major mRNA species in different cells (Fig. 3). Inspection of the autoradiographs reveals several HIB resistant mRNA translation products: in MPC-11 cells (channels A & B), these include in addition to the L (23,000) and H (55,000) polypeptides, a peptide of 42,000 molecular weight and the histones (11,000 to 14,000). The translation of these non IgG mRNAs in other cell lines is also resistant to HIB, HeLa (channels D-F), L-929 (channels G-I) and BHK-21 (channels J-L). In contrast, the synthesis of two major cellular proteins, actin (41,000) and myosin (200,000) is highly sensitive to HIB.

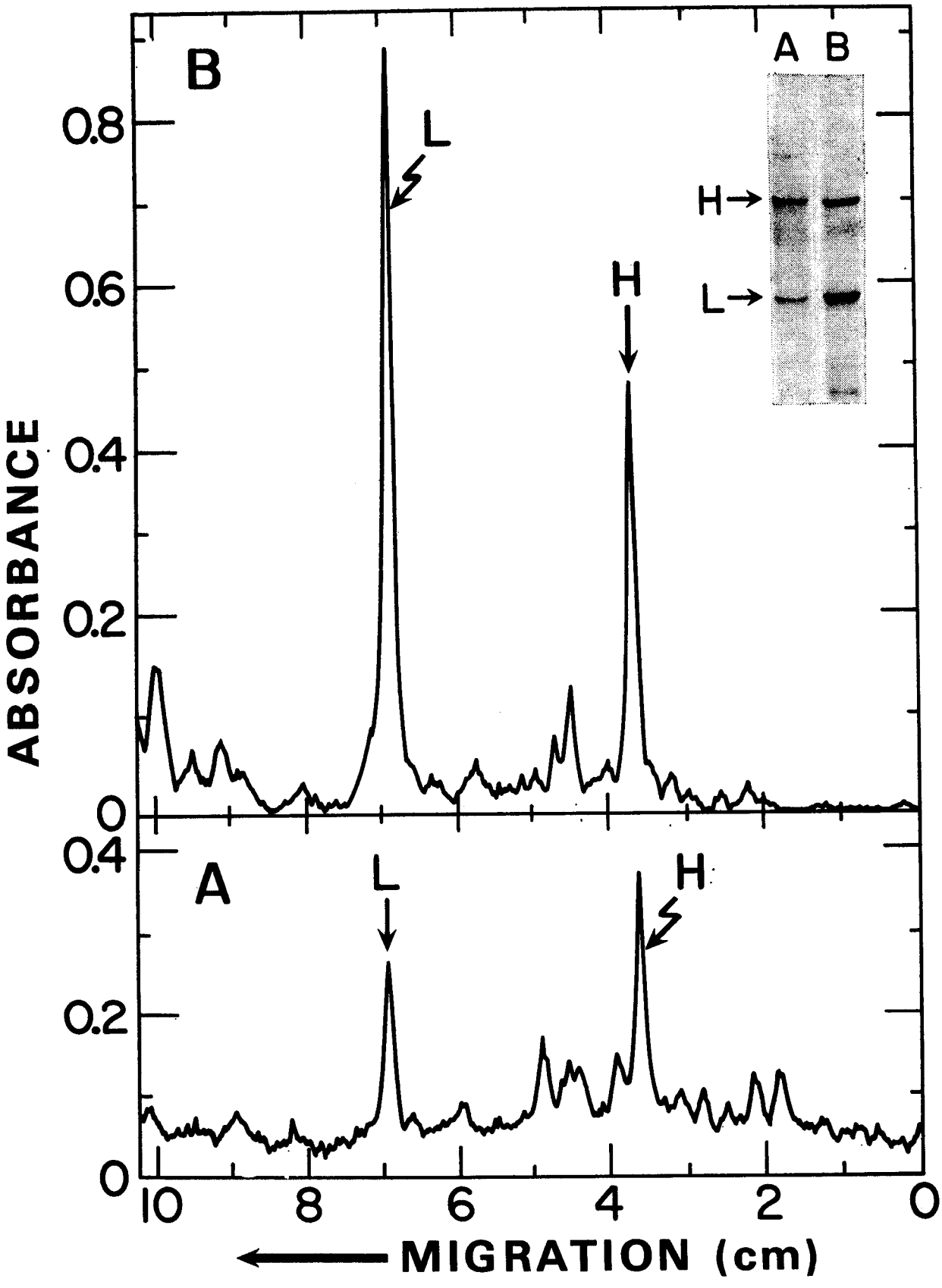

Fig. 2: Distribution of [^{35}S]methionine incorporation at isotonic and hypertonic conditions as revealed by absorbance scanning of gel autoradiographs.

An equal number of counts from the cytoplasmic extracts prepared from cells pulse

A further refinement of this technique will allow one to estimate the relative translational efficiencies of individual cellular mRNAs.

Comparison of the relative synthesis of IgG and non-IgG polypeptides under conditions of reduced rates of polypeptide chain initiation and of polypeptide chain elongation

As previously shown in Figs. 2 and 3, the distribution of amino acid incorporation into IgG and non-IgG polypeptides is significantly influenced by the rate of polypeptide chain initiation. Table 3 shows a more detailed analysis of the change in the relative synthesis of IgG L, H and non-IgG polypeptides with increasing concentration of excess NaCl or emetine (Lodish, 1971), an hinhibitor of peptide chain elongation. Notice that as the concentration of NaCl is increased, the overall incorporation of [^{35}S]methionine decreases while the percent of [^{35}S]methionine incorporation which is associated with the IgG polypeptides increases dramatically; similarly the ratio of L/H (after correction for the relative amounts of methionine residue in the two polypeptides) increases from 1.6 to 3.6 when protein synthesis is reduced by $\sim$95 %.

In contrast, the percent of [^{35}S]methionine incorporation which is IgG-specific, decreases when polypeptide chain elongation is reduced by exposure of cells to emetine. There is also a slight increase in the L/H ration (1.55–1.92) with increasing concentration of emetine. Under conditions where the elongation step in translation is rate limiting, the relative synthesis of individual polypeptides is thought to be proportional to the relative amounts of the mRNA coding for these peptides (Lodish, 1974; MacDonald and Gibbs, 1969). Accordingly, these results would indicate that this cell line contains twice the amount of message for L chain than for H chain. Thus, the value of 1.6–1.7 for the ratio of L/H at isotonic conditions indicates that L chain is synthesized under this condition at a lower rate than expected from the relative amounts of L and H mRNAs. This observation is in accord with several models proposed for the regulation of mRNA translation (MacDonald, Gibbs and Pipkin, 1968; MacDonald and Gibbs, 1969; Lodish, 1974), which predict that the translation of such mRNAs as the L chain mRNA which possesses a high rate constant for initiation is hindered in the elongation step by an increased density of ribosomes on the mRNA molecule under conditions where the overall rate of peptide chain initiation is high.

Comparison of the effects of HIB and virus infection on the relative synthesis of IgG and non-IgG proteins in myeloma cells

Virus induced suppression of host mRNA translation might be exerted by alterations of the protein synthesizing mechanism which allows ribosomes to specifically interact only with viral mRNA (Matthews *et al.*, 1973; Wright and Cooper, 1974). Alternatively, it was suggested that virus induced suppression of host mRNA translation, like HIB, acts at the level of peptide chain initiation (Liebowitz and Penman, 1971) and that an indiscriminate reduction in the rate of peptide chain initiation would provide a translational advantage for viral mRNA (Nuss,

labeled in isotonic (lower panel A) and hypertonic (140 mM excess NaCl) (upper panel B) medium were applied to the two gel channels. The actual autoradiographs are shown in the upper right hand corner of panel B.

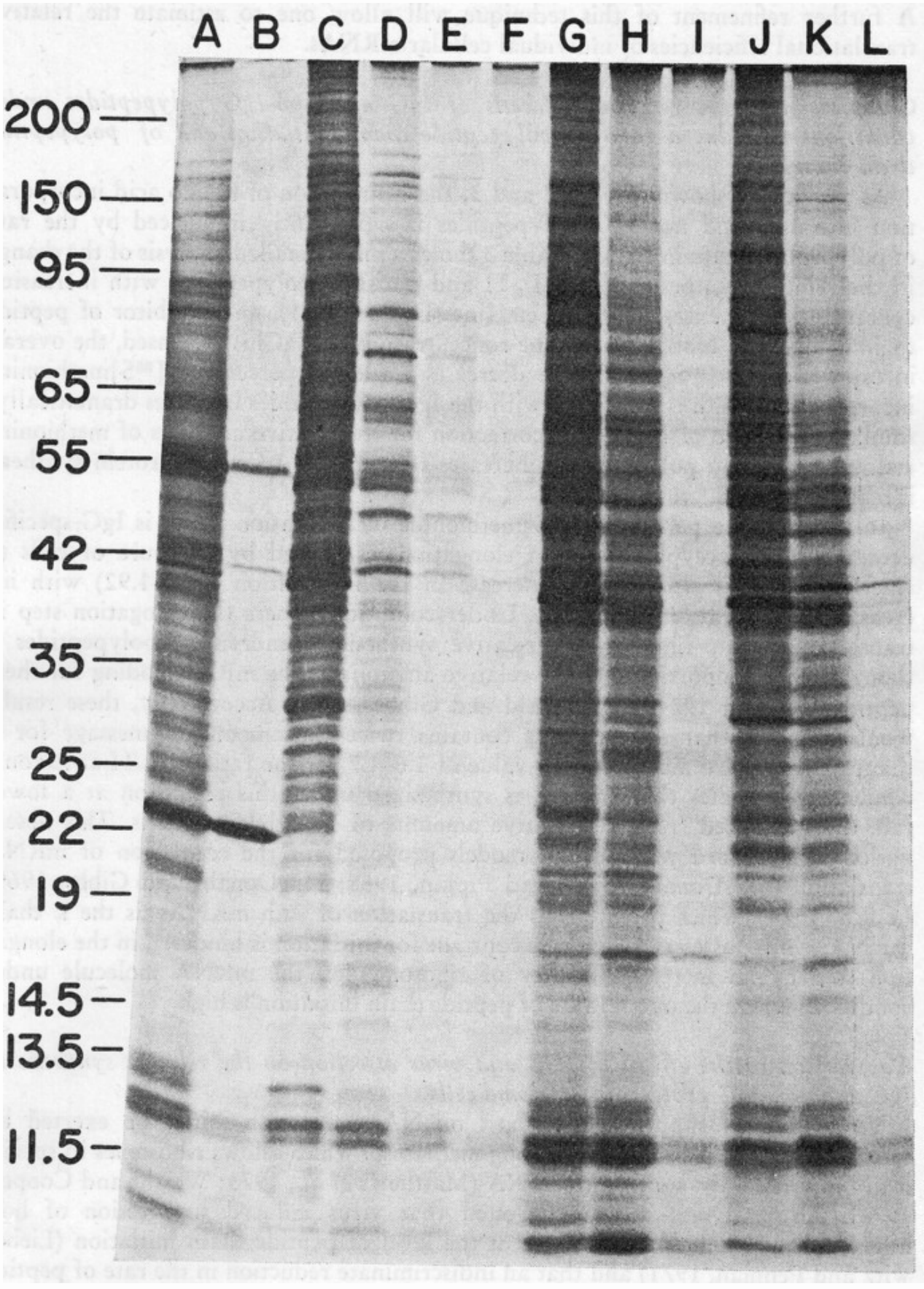

Fig. 3: HIB induced changes in the relative synthesis of individual cellular proteins.

Protein synthesis in various tissue culture cells was analyzed by isotope labeling and acrylamide gel electrophoresis under control and hypertonic conditions as described in Materials and Methods.

Myeloma cells, channel A and B, HeLa cells (C–F), L929 cells (G–I) and BHK21 cells

Table III: Effect of HIB and Emetine on the synthesis of IgG and non-IgG proteins in myeloma cells

[^{35}S]methionine incorporation % of Control		HIB				Emetine			
		% of [^{35}S] Protein as			Ratio	% of [^{35}S] Protein as			Ratio
HIB	Emetine	L+H	L	H	L/H	L+H	L	H	L/H
100	100	17.4	6.9	9.5	1.60	16.2	6.7	9.5	1.55
92		17.6	7.7	9.9	1.71				
	77					16.3	7.1	9.8	1.60
64	57	19.4	8.6	10.8	1.76	15.7	6.9	8.8	1.73
45	35	22.9	10.4	12.5	1.82	15.7	7.0	8.0	1.78
20	26	26.9	14.0	12.9	2.38	14.7	7.0	7.7	2.00
11	–	30.5	17.8	12.7	3.08		–	–	–
6	6	34.8	21.6	13.2	3.60	11.3	5.6	6.3	1.92

Table 3: MPC-11 plasmacytoma cells at a cell density of 3×10^6 cells/ml in 3.8 ml of serum-free Joklik's MEM containing 1/20 the normal concentration of methionine plus 25 mM HEPES, pH 7.4 were incubated in the presence or absence of extra NaCl or emetine for 15 min at which time each sample received 100 µCi of [^{35}S]methionine (NEN) and incubation was continued for an additional 15 min. After proper chase, cytoplasmic extracts were prepared and analyzed by polyacrylamide-SDS-gel electrophoresis as described previously (Nuss, Oppermann and Koch, 1975). The range of NaCl used to inhibit protein synthesis was from 0 to 140 mM excess while emetine was used at concentrations up to 400 ng/ml. As an indication of the levels of radioactivity measured in these experiments, approximately 170,000 cpm were recovered from the total control gel channel. The data are presented as the percent of total [^{35}S]methionine incorporation which was found in the L and/or H bands and the ratio of radioactivity found in the L and H bands after correcting for the relative number of methionine residues in these respective polypeptides.

Oppermann and Koch, 1975). If this suggestion is correct, the synthesis of these cellular proteins which are resistant to HIB should also be resistant to virus-directed suppression. Since IgG synthesis in myeloma cells is relatively resistant to HIB, this cell system offers a special advantage for the analysis of the mode of inhibition of protein synthesis by virus infection. We, therefore, compared the effects ob HIB and virus infection on the relative synthesis of IgG and non-IgG proteins in myeloma cells.

(J–L). The first channels (A, C, G, and J) represent peptides labeled under isotonic conditions.

Extra NaCl was added to all other cell cultures in the following amounts 100 mM (D), 120 mM (E, H, K) and 140 mM (B, F, I and L). All cultures with extra NaCl received 2 X the amount of ^{35}S-methionine added to the isotonic control.

The electrophoretic mobilities of proteins was compared to the migration of proteins with known molecular weights: Muscle myosin, E. *coli* DNA dependent RNA polymerase subunits β, β', σ and α, bovine serum albumin, ovalbumin, myeloma IgG heavy (H) and light (L) chain, purified poliovirus coat proteins VP1, 2 and 3 and RNase. The migration of polypeptides in the discontinuous buffer systems does not accurately reflect their molecular weights. The assigned molecular weights serve merely for orientation on the autoradiographs.

Table IV: Effect of HIB and VSV infection on the synthesis of total protein and of IgG peptide chains in myeloma cells

[^{35}S]methionine incorporation % of Control		HIB		VSV	
HIB	VSV	% of [^{35}S] Protein as L	Ratio L/H	% of [^{35}S] Protein as L	Ratio L/H
100	100	6.9	1.60	6.4 ± 0.8	1.8 ± 0.1
64	50 ± 3	8.6	1.76	9.7 ± 0.0	2.3 ± 0.0
45	38 ± 1	10.4	1.82	10.9 ± 1.4	2.7 ± 0.3
20	17 ± 2	14.0	2.30	10.7 ± 1.6	3.0 ± 0.5
11	8	17.8	3.08	12.5	3.7

Table 4: This table shows the change in the percent of total ^{35}S-methionine incorporation associated with the L chain and the change in the L to H ratio in uninfected cells with increasing concentrations of excess NaCl (as in Table 3), and in VSV infected cells with time after infection at isotonic conditions. Adsorption of VSV, serotype Indiana (Mudd and Summers, 1970) was performed at room temperature for 20 min with MPC-11 cells suspended at a density of 1 x 10^7 cells/ml in serum-free Dulbeco's MEM. Following adsorption, the cell suspension was diluted to a density of 1 x 10^6 cells/ml in Dulbecco's MEM supplemented with 10 % horse serum and incubated at 37 °C in 250 ml tissue culture flasks (Falcone). The data presented in the column entitled VSV give the percent incorporation into host specific polypeptides at different time after infection (0 to 5 hrs). The data with infected cells represent the average of two experiments.

Infection of myeloma cells with VSV results in a rapid inhibition of total protein synthesis (Nuss and Koch, 1976a) and in alterations in the distribution of labeled amino acids into L, H and non-IgG polypeptides similar to that observed following exposure of uninfected cells to HIB (Table 4). These results support our proposal that a major event in the strategy of virus induced suppression of host mRNA translation is an indiscriminate reduction in the overall rate of peptide chain initiation (Nuss, Oppermann and Koch, 1975; Nuss and Koch, 1976a).

Discussion

Peptide chain initiation is inhibited selectively by an increase in the tonicity of the growth medium (Saborio, Pong and Koch, 1974). The application of this method to a study on protein synthesis in several RNA virus infected and uninfected cells has revealed that all viral mRNA species possess a greater ability to intitaiate translation than cellular mRNAs. Accordingly, viral protein synthesis can be unmasked from cellular protein synthesis by a brief exposure to the hypertonic initiation block (HIB) (Nuss, Oppermann and Koch, 1975). The translational efficiency for individual cellular mRNAs varies greatly. Inhibition of peptide chain initiation by HIB in myeloma cells increases the percent of [^{35}S] incorporation into L + H chains from, 17.5 % to 34.8 %, inhibition of peptide chain elongation by emetine reduces this percentage to 11.3 % (Table 3). These results indicate that translational control is operative in tissue culture cells also under standard growth conditions, however only to a limited extent.

Competition between viral and host mRNA for ribosomes in virus-infected cells is likely to have an impact on host protein synthesis. Competition alone may be sufficient to account for the shift from host to viral protein synthesis in virus infected cells where total protein synthesis remains constant during the virus replication cycle. In poliovirus infected HeLa cells (Nuss, Oppermann and Koch, 1975) and in VSV infected myeloma cells (Nuss and Koch, 1976c), however, total protein synthesis is inhibited at times prior to the detectable synthesis of viral RNA and viral proteins. The observation that the selective inhibition of peptide chain initiation by HIB results in the potentiation of the virus induced suppression of host protein synthesis at early times in the infectious cycle suggests that virus directed suppression may also be exerted at the level of peptide chain initiation.

Based on these observations, we would predict that the synthesis of those cellular proteins which show resistance to HIB should also be relatively resistant to virus directed suppression and those cellular proteins which are very sensitive to HIB should also be preferentially affected by virus infection. We tested this prediction in two virus-host cell systems a) in VSV infected myeloma cells, b) in poliovirus infected HeLa cells. The synthesis of IgG peptides, which make up 15 to 20 % of total protein synthesis in myeloma cells are relatively resistant to HIB, and also resistant to inhibition by VSV infection (Nuss and Koch, 1976c). The synthesis of actin in HeLa cells is considerably more sensitive to HIB and inhibition of protein synthesis exerted by infection with poliovirus than is the synthesis of other cellular proteins (Oppermann and Koch, unpublished results). These results support our previous proposal (Nuss, Opermann and Koch, 1975), that virus directed suppression of host protein synthesis need not involve virus specific or virus induced-factor(s) which possesses the capacity to actively discriminate between viral and host mRNA, but could perform its function by indiscriminately lowering the overall rate of peptide chain initiation for the translation of all mRNA.

Although the exact mechanism whereby virus infection or exposure of cells to excess NaCl inhibits peptide chain initiation is not clear, one straight forward explanation is that they both lower the rate of peptide chain initiation by interfering with the association of ribosomes and mRNA. In both instances this inhibition of peptide chain initiation might proceed by triggering existing cellular control mechanisms. It was previously suggested that protein synthesis in animal cells can be regulated by membrane mediated events (Pong, Nuss and Koch, 1975). This view is supported by the observation that several inhibitors of *in vivo* peptide chain initation such as increased medium osmolarity, TPCK, and cytochalasin B, show little effect on this process in cell-free extracts prepared from HeLa cells or L cells. The membrane-mediated regulation of protein synthesis may involve changes in cyclic nucleotides (Pong, Nuss and Koch, unpublished data) or changes in the membrane structure. Alterations in the structure of the cell membrane by exposure to proteolytic enzymes have been correlated with changes in surface adhesion of cells and in the growth rate of cells (Burger, 1970; Sefton and Rubin, 1970; Srere, 1974). Indeed, we observed that addition of protease-released membrane glycopeptides (10 to 100 μg/ml) to suspended HeLa cells results in a rapid inhibition of cellular protein synthesis (Koch, Kubinski and Koch, 1974). The protease-released membrane components also inhibit protein synthesis in cell-free extracts (Fisher and Koch, unpublished results).

Under optimal growth conditions, the relative synthesis of proteins in tissue culture cells appears to be regulated mainly, but not exclusively, by differential transcription of mRNA. However, under several natural (Fan and Penman, 1970) and experimental conditions which restrict growth and/or reduce the overall rate of peptide chain initiation, additional regulatory mechanisms on the translational level become decisive. We propose that control of protein synthesis on the translational level is amplified when the overall rate of complex formation between ribosomes and mRNA is indiscriminately lowered. When peptide chain initiation becomes rate limiting, then all mRNAs with high binding affinities to ribosomes are preferentially translated and every mRNA species exhibits a characteristic ability to initiate translation. Consistent with this interpretation is the recent observation that the binding affinities of synthetic oligonucleotides to ribosomal subunits depend on their nucleotide sequence composition, and their structure at the 5' end (G. Both, Y. Furuichi, S. Muthukrishnan, and A. Shatkin, manuscript in preparation). The differential affinities of individual mRNAs species for ribosomes might be a decisive factor in the regulation of the growth cycle. The techniques described here provide a suitable tool to investigate this possibility.

Infection by RNA-viruses is favored when the rate of peptide chain initiation is reduced (Koch, 1973). Since unfavorable growth conditions – especially amino acid starvation – result also in inhibition of peptide chain initiation, they are expected to sensitize cells for virus infection. This view is supported by the recent observation that infection of tissue culture cells by certain picornaviruses is severely inhibited by addition of nonessential amino acids to the growth medium (Verhagen, pers. commun.). Susceptibility to virus infection in animals and men might be influenced by similar mechanisms.

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