take of sodium by the gills of goldfish in fresh water.

In an attempt to reconcile the data currently available on the ion-regulatory activity of the caudal neurosecretory system in teleosts, we suggest that the urophysis may release two neurohormones: one which favors active ion transport in the normal direction and another that either inhibits the normal active transport or stimulates active transport in the opposite direction in response to abnormal osmotic circumstances. We are currently obtaining electrophysiologic data, of the kind described herein, on the effects of altering the internal osmotic environment by intravenous injections of saline solutions of various kinds. Results to date indicate the presence of two types of caudal neurosecretory fibers in individual *Tilapia*. One type, corresponding to the fibers stimulated by a hypertonic external environment, is depressed by intravenous injection of sodium-free hypotonic solution and stimulated by a subsequent injection of saline containing ten times the normal sodium chloride concentration; the other type of fiber shows exactly the opposite responses. It is possible that the first type of fiber is associated with an increase in total ion flux in a direction opposite to normal, whereas the second enhances normal osmoregulatory processes.

However, the situation regarding ion regulation by the urophysis must remain only conjectural for the present. It is assumed that impulse conduction by neurosecretory fibers (see *6*, *7*) bears an essential relation to neurohormone release, but this logical assumption has yet to be firmly established (*8*).

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25 July 1963

## Translational Control of Protein Synthesis in a Cell-Free System Directed by a Polycistronic Viral RNA

Abstract. *Observations with RNA isolated from the RNA virus indicate that regulation of protein synthesis occurs during translation. At least three electrophoretically separable proteins, one of which lacks histidine, appear as a result of adding purified viral RNA to a cell-free extract of* Escherichia coli. *This establishes that the RNA contains at least three cistrons. Comparison of the kinetics of appearance of the proteins that do and do not contain histidine reveals a control mechanism which determines the temporal order and the frequency of translation of each cistron. Such translational controls at the stage of message use provide additional devices for regulating protein synthesis which can supplement those functioning at the transcription step where genetic messages are produced.*

Currently accepted views of how information flows from the genome to the protein-synthesizing machines suggest two possible sites at which controls can be inserted. One is at the step (transcription) which produces the complementary RNA copies which serve as genetic "messages." The other would be where the genetic "messages" are translated into the protein molecules. Regulation of both production and use of message are not mutually exclusive and both mechanisms can, in principle, coexist. Recent experiments with the "gal" (*1*) and "lac" (*2*) loci of *Escherichia coli* have shown that inducers of specific enzyme synthesis stimulate the accumulation of the particular messages complementary to the homologous genetic regions. These results suggest a control of gene transcription. We now describe experiments from RNA from an RNA virus in a cell-free system showing that there is regulation at the point of protein synthesis.

An RNA molecule which can be translated into two or more proteins may be referred to as a "polycistronic" message. This concept immediately raises the possibility of regulated use in terms of the order and relative frequency of translation of the component cistrons. The RNA message fraction has been shown to contain molecules big enough to act as a code for several proteins (*3*, *4*), an observation confirmed with other systems (*5*, *6*). It has been proposed (*7*) that such polycistronic messages could arise from the continuous transcription of contiguous cistrons belonging to the same "operon." A simple physical basis is thus provided for the "operon" concept (*8*).

The RNA viruses guarantee the existence of polycistronic molecules and also provide a convenient material for their experimental analysis. Data on mutations of tobacco mosaic virus (*9*) show that viral RNA determines the amino acid composition of the coat protein. Further, it seems very probable that replication of viral RNA is mediated by an RNA-dependent RNA polymerase (*10*, *11*). Suggestive supporting evidence (*12*, *13*) has recently appeared without, however, proof of RNA dependence. Available evidence (*14*, *15*) makes it highly unlikely that a polymerase of this sort already exists in uninfected cells. The RNA polymerase discovered by August *et al.* (*16*), is not a likely candidate for fabricating specific heteropolymers. While it is stimulated by RNA, it apparently cannot catalyze the synthesis of anything but polyadenylate and the end product is uninfluenced by the "primer." We therefore predicted (*10*) that the structural program of the new polymerase is coded in the RNA of the virus particle. From these arguments, a viral genome must contain cistrons for at least two proteins, and hence all viral RNA molecules must be polycistronic. We present direct supporting evidence for this conclusion.

If a new polymerase must be synthesized before RNA replicas appear, the incoming viral RNA must obviously serve as a message and must furthermore be conserved while being translated into protein (*10*). The prediction of conservation was confirmed (*17*) through the use of double labeling ($N^{15}$ and $P^{32}$) and by showing that the two isotopes can be recovered in the same RNA strands at the end of a complete lytic cycle.

Our principal concern here is with the conclusion that viral RNA must contain a device which dictates the number of times a particular cistron is

translated. This deduction follows as a numerical consequence of the number of coat protein subunits needed to complete a virus particle compared to other proteins specifically required for viral maturation. A specific example is the RNA bacteriophage (MS$\phi$2) which is similar in many of its properties to the f2 bacteriophage (*18*). Its RNA is approximately $1 \times 10^6$ (*19*) in molecular weight and supplies the code for at least three proteins. One is the coat protein, another an RNA-dependent polymerase (*20*), and the function of the third has not been identified. The molecular weight of the coat-protein subunit is about 20,000, and there are approximately 200 such subunits per particle. Since the normal burst size of this virus is between 1000 and 10,000 per cell, between $2 \times 10^5$ and $2 \times 10^6$ coat-protein molecules are made per cell per lytic cycle. It is difficult to imagine that an equal number of polymerase molecules are synthesized. The same argument would apply to any other enzyme or protein not included as a component of the mature particles. This situation virtually demands a mechanism which insures that the cistron for the coat protein be translated more times than those which correspond to some of the other proteins. It seems likely that this device would be built into the RNA molecule and could operate even outside the cell.

We now raise the problem of whether evidence for a control mechanism of this sort can be detected in an in vitro system. Clearly one requirement for an experimental resolution is the ability to distinguish the coat protein from the the others. This possibility is provided by the RNA bacteriophage MS$\phi$2 since its coat protein lacks histidine (*19*). Consequently, histidine can be employed as a marker to detect the appearance of histidine-containing proteins synthesized under the direction of the viral RNA. With this device one can readily compare the kinetic details of the appearance of coat and non-coat proteins.

Nathan *et al.* (*21*), using the cell-free system of Nirenberg and Matthaei (*22*), showed that the RNA of f2 stimulates the incorporation of amino acids into proteins similar in their peptide chromatographic and electrophoretic patterns to the coat protein. Also, proteins which contain histidine are formed. We have confirmed these findings with RNA isolated from MS$\phi$2.

Comparison of the kinetics and the time of entry of histidine and some other amino acid commonly contained in protein should provide definitive information on the presence or absence of order and regulation in the synthesis of the proteins directed by the viral RNA.

In thinking about possible outcomes, one must recognize that order versus randomness in the translation of a set of cistrons involves questions of time, frequency, and the relation of these to location in the RNA molecule. Thus, consider the coat protein cistron and two others arranged in some sequence. The following possibilities can be imagined: (i) no preferred order of translation and the frequency of translation is equivalent for all three cistrons; (ii) a preferred order and the translation frequency is equivalent for all three cistrons; (iii) no preferred order but the number of translations per unit time is considerably greater for the coat proteins; (iv) a preferred order and the translation frequency for the coat-protein cistron is considerably higher than for the others.

Mechanisms similar to i and ii are unlikely in the cell since they predict that equal numbers of all three types of protein molecules would be synthesized per unit time. They could, however, occur in a cell-free system, and they can be distinguished only by limiting the experiment to a time interval comparable to one translation of each of the cistrons. In any long-term experiment both mechanisms would have similar kinetics of appearance for all three proteins. From the third mechanism one would predict that proteins containing histidine should begin appearing at the same time as the coat protein which does not contain histidine. However, the relative rate of formation of the coat protein would be higher and would remain constantly so throughout the period of active synthesis. Finally, the fourth mechanism would imply a break in the rate of appearance of histidine-containing pro-

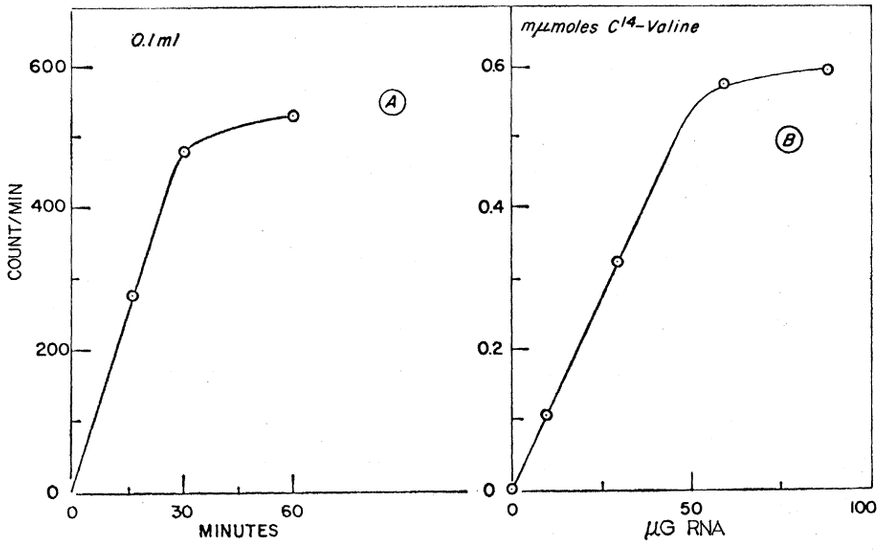


Fig. 1. Characteristics of amino acid incorporation in cell-free extracts of *E. coli*. General reaction conditions and method of measuring incorporation: The volume of the reaction mixtures was 0.25 ml, containing 0.04*M* tris HCl, *p*H 7.8; 0.011*M* magnesium acetate; 0.008*M* $\beta$-mercaptoethanol; 0.032*M* KCl; 0.002*M* ATP; 0.0004*M* GTP; 0.012*M* phosphoenolpyruvate; 50 $\mu$g of pyruvate kinase per milliliter; $4 \times 10^{-5}$ mole of each of the 20 amino acids, and $\frac{1}{5}$ volume of the supernatant obtained from centrifugation at 30,000*g*. Reactions were carried out at 35°C, and all mixtures were incubated for 20 minutes before labeled compounds or RNA fractions were added. Incubations were terminated by adding 3 ml of 7 percent PCA, and then heated at 90°C for 15 minutes. The resulting precipitates were centrifuged, dissolved in 0.5 ml of 0.1*M* NaOH, and reprecipitated with 4 ml of 10 percent TCA. The precipitate was then transferred to a Millipore filter and washed with three 10-ml portions of 10 percent TCA. After drying, the radioactivity was assayed in a liquid scintillation counter (*23*). (*A*) Kinetics of incorporation: Uniformly labeled amino acids, $4 \times 10^5$ count/min per milliliter (0.166 mc/mg) were added at zero time with 100 $\mu$g of RNA of the purified phage of MS$\phi$2 (*21*). At intervals, 0.1-ml samples were removed and treated as described. (*B*) Effect of RNA concentration: A series of the aforementioned reaction mixtures (0.25 ml) were set up and the indicated amounts of RNA (micrograms) added along with $C^{14}$-labeled valine, $4 \times 10^5$ count/min (100 $\mu$c/$\mu$mole). The reaction was allowed to proceed for 60 minutes at 35°C, and then the mixture was prepared for radioactive counting.

teins. The position of the break would depend on the temporal order in which the cistrons become active.

In standardizing our experiments, active extracts were always prepared from cells of *Escherichia coli* strain B growing in the logarithmic phase on a modified Penassay medium (*23*). This strain cannot be infected with the RNA bacteriophage MS$\phi$2. After washing with cold buffer the cell paste was ground with 2.5 times its weight of alumina and extracted with 2.5 volumes of 0.01*M* tris, *p*H 7.8, containing 0.01*M* magnesium acetate, 0.06*M* KCl, 0.006*M* $\beta$-mercaptoethanol, and 5 $\mu$g of deoxyribonuclease per milliliter. The resulting extract was cleared by centrifugation at 10,000*g* for 10 minutes and the supernatant was centrifuged at 30,000*g* for 30 minutes. The final supernatant was dialyzed for 4 hours against 0.01*M* tris, *p*H 7.8 containing 0.01*M* magnesium acetate, 0.06*M* KCl, and 0.006*M* $\beta$-mercaptoethanol. Such extracts had an optical density at 260 m$\mu$ of 180 and could be stored frozen for several months without losing activity. Each reaction mixture contained $\frac{1}{5}$ volume of extract. All other experimental details are described in the figure legends.

The kinetics of incorporation of amino acids by such extracts are shown in Fig. 1*A* and indicate that incorporation is linear for about 30 minutes, and then declines. Further, the system becomes saturated (Fig. 1*B*) when the reaction mixture contains approximately 50 $\mu$g of phage RNA. From these results comparisons of rates of incorporation of different amino acids are best confined to the linear period.

In the comparison of the incorporation of histidine with some other amino acids, a number of precautions must be exercised. To obviate irrelevant kinetic differences which might occur from one preparation to another, two different isotopes (for example $H^3$ and $C^{14}$) have been used to identify the two amino acids being compared. The incorporation of both in the same reaction mixture can then be followed by measuring radioactivity in a liquid-scintillation spectrometer and, thus, each amino acid serves as an internal control of the other. Under these circumstances observed kinetic differences can be accepted as real and interpretable. Further, the specific activities of the amino acids used must be adjusted to the extent of their incorporation so that the sensitivities of the observations of the two are comparable. Finally, background activity, unassociated with synthesis specific to viral RNA, can be eliminated by incubation of the ribosome preparation (in our experiments, 20 minutes) with all necessary supplements prior to adding the viral RNA and the labeled amino acids.

Because of their central importance, two deductions made in the introductory paragraphs merit direct test to insure that our data are relevant to the translation of polycistronic RNA. First it must be shown that the viral RNA added, and not some polynucleotide synthesized under its direction, is actually serving as the message and second that it is polycistronic in vitro.

To check the first point, the simultaneous incorporation of $UTP^{32}$ (uridine triphosphate with $P^{32}$ in the nucleotide phosphorous) into polynucleotide and $C^{14}$ labeled valine into protein was com-

Table 1. Effect of viral RNA on the incorporation of $P^{32}$-labeled UMP and $C^{14}$-labeled valine. Details of the reaction conditions and the common supplements are described in the first section of Fig. 1. When included, 160 $\mu$g of purified RNA (per milliliter) from MS$\phi$2 was added. The final specific activity of the $C^{14}$-valine was 10 $\mu$c/$\mu$mole and 14 m$\mu$mole of $UTP^{32}$ at $6 \times 10^7$ count/min per micromole was also added. After 40 minutes at 35°C, the incubation mixture was divided into two equal portions. $P^{32}$ was counted in the precipitate formed in cold 10 percent trichloroacetic acid (TCA), and after washing with the same acid on Millipore filters. $C^{14}$ was counted after the usual hot perchloric acid (PCA) extraction, solution, reprecipitation, and so forth, described for Fig. 1.

| Time (min) | Viral RNA | Incorporation | | | |
|---|---|---|---|---|---|
| | | $UMP^{32}$ | | $C^{14}$-valine | |
| | | count/min | m$\mu$mole | count/min | m$\mu$mole |
| 0 | | 71 | | 22 | |
| 40 | − | 1193 | 0.018 | 46 | 0.006 |
| 40 | + | 866 | 0.014 | 2592 | 0.64 |

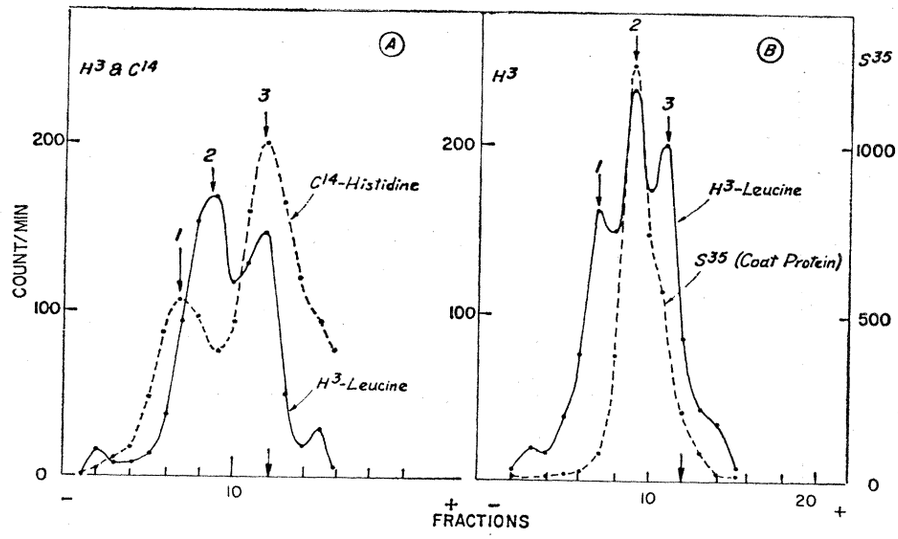


Fig. 2. Electrophoretic separation of components synthesized under the influence of viral RNA. (*A*) One-half milliliter of reaction mixture, as described in Fig. 1, was incubated for 20 minutes; $H^3$-labeled leucine (final specific activity 2.8 mc/$\mu$mole), and $C^{14}$-labeled histidine (at 22 $\mu$c/$\mu$mole) along with MS$\phi$2-RNA (160 $\mu$g/ml) were added. After 60 minutes, 0.3 ml of the reaction mixture was adjusted to 5*M* urea, 0.01*M* EDTA and 0.3*M* sodium acetate at *p*H 5.6. The material was then applied at the position indicated by the arrow to a 2 by 36-cm column of 40 g of "geon" (B. F. Goodrich Chemical Co.), and electrophoresis in 0.03*M* sodium acetate, *p*H 5.6; 5*M* urea; 0.01*M* EDTA was then instituted at 500 volts and 10 ma. After 24 hours, 2-cm sections of the geon column were cut out and the protein was eluted with 3 ml of the urea-EDTA-acetate buffer. Carrier protein (1 mg of serum albumin) was added to each fraction. Labeled material was precipitated with 6 percent TCA containing 0.5 percent phosphotungstic acid, washed on Millipore filters, and counted in a liquid scintillation spectrometer. (*B*) Everything is as described for *A* except that labeling was only with $H^3$-labeled leucine and $S^{35}$-labeled coat protein was added to the mixture prior to application to column. The $S^{35}$-labeled virus particles were prepared as described previously (*15*) for $P^{32}$-labeling except that 7 mc of $S^{35}$ as $SO_4^{--}$ was included, the $MgSO_4$ in the medium being adjusted to 0.001*M*. The phage protein was purified by the acetic acid procedure (*24*).

pared in the presence and absence of viral RNA. Standard conditions, routine for all experiments, were used with extracts from noninfected cells and where the supplements never contained CTP (cytosine triphosphate). RNA dependent polymerase is absent from cells which have not been exposed to virus (*20*) and, furthermore, its activity requires the presence of all four riboside triphosphates. Table 1 summarizes the results obtained as determined by incorporation of $UTP^{32}$ into acid-insoluble material. While there is detectable incorporation, it is minute (less than 0.02 m$\mu M$) and the amount of apparent polynucleotide synthesized is not increased by the addition of viral RNA. Further, the small amount of incorporation of $UMP^{32}$ is completely uncorrelated with protein-synthesizing activity. The same reaction mixture is virtually completely dependent on added viral RNA for incorporation of $C^{14}$-labeled valine (Table 1). The observed stimulation of amino acid incorporation is apparently not accompanied by detectable synthesis of RNA specifically stimulated by the addition of viral RNA. These results are clearly consistent with the conclusion that the added RNA is serving directly as the translatable message and is responsible for the observed stimulation of amino acid incorporation.

That the viral RNA is polycistronic would receive direct support by the demonstration that histidine is incorporated into a protein (or proteins) separable from the coat protein. The insolubility of the coat protein in the usual buffers, and the tight ribosomal association of the histidine-containing proteins synthesized were eliminated by the addition of urea (5*M*) and versene (0.01*M*). The synthesized proteins were then separated by electrophoresis on columns of "geon" (Fig. 2*A*).

It is clear from the profiles of Fig. 2*A* that there are three major components, indicated by the numbers 1, 2, and 3. The relative distributions of the histidine and leucine suggest that component 2 is either low in, or completely lacks, histidine and may be identified with the coat protein. That this is the case is strongly supported by the results of the experiment described in Fig. 2*B*. Here the product of a reaction mixture labeled for 60 minutes with tritiated leucine was mixed with $S^{35}$-labeled coat protein purified from isolated viral particles. The resulting mixture was then subjected to electrophoretic separation. The $S^{35}$-labeled protein moves in the same position as peak 2. These results would appear to establish that MS$\phi$2 viral RNA is polycistronic and contains cistrons which code for at least three protein components. Two of the components clearly contain histidine and the third is identifiable with the coat protein which lacks this amino acid.

There is too much contamination of one peak by another in Fig. 2 to permit a meaningful approximation of the relative amounts of each component formed. An estimate can, however, be attained from a comparison of the incorporation of molar amounts of histidine with that of some other amino acid. In a typical reaction run for 60 minutes, 0.77 m$\mu$mole of valine and 0.02 m$\mu$mole of histidine were incorporated. Thus for every 38 moles of valine, only 1 mole of histidine is used. On the basis of the 6 to 1 ratio of valine to histidine commonly found in protein, one would estimate that the ratio of the masses in peaks 1, 2, and 3 is approximately as 1:12:1. If the comparison is made in terms of number of molecules, the ratio is likely to increase further in favor of the coat protein in view of its very small molecular weight. In any case, the data indicate preferential synthesis of a protein which does not contain histidine.

With the information available one can use the system to analyze questions of randomness and frequency of the translation mechanism. We now turn to the problem of sequential appearance of the classes of proteins which can be identified with the aid of histidine. When one compares the kinetics of entry into protein of $H^3$-histidine and $C^{14}$-valine, results are obtained as described in Fig. 3*A*. There is clearly no detectable lag in the time of entry of $C^{14}$-valine into protein precipitable by hot acid. It is equally evident that there is a distinct lag amounting to about 6 minutes, before the appearance of proteins containing tritiated histidine. Similar experiments were performed with other amino acids and with the radioactive labels reversed (for example, $H^3$-leucine and $C^{14}$-histi-

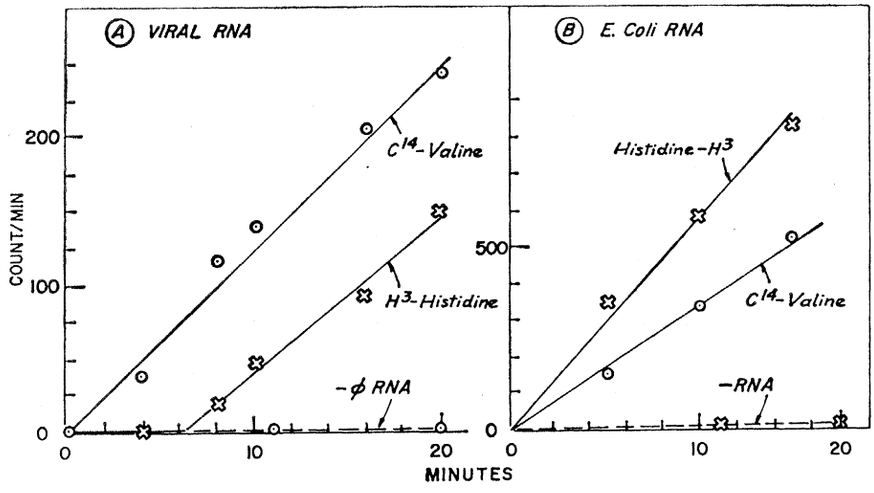


Fig. 3. Kinetics of incorporation of histidine labeled with $H^3$ and valine labeled with $C^{14}$. (*A*) One milliliter of the standard reaction mixture (Fig. 1), 1.0 ml, was incubated for 20 minutes and $H^3$-labeled histidine (final specific activity 1.1 mc/$\mu$mole and $C^{14}$-labeled valine (final specific activity 8 $\mu$c/$\mu$mole were introduced. At the same time, 160 $\mu$g of RNA purified from MS$\phi$2 phage was added to one and omitted from a control. The specific activities chosen were determined by the proportion of the two amino acids incorporated and the efficiency of counting of $C^{14}$ and $H^3$. The points represent counts per minute ($H^3$ or $C^{14}$) in each 0.1 ml removed at the indicated time intervals. Only the counts for $C^{14}$ are shown in the control lacking RNA since those for the $H^3$ were equally negligible. Each sample was prepared for counting as described in Fig. 1. Window settings for double counting were such that cross-channel contamination was 25 percent in the direction of $C^{14}$ to $H^3$ and 5 percent in the reverse direction. The same settings yielded similar results with $S^{35}$ and $H^3$. (*B*) To the same reaction mixture that was used for the experiment in Fig. 3*A*, 1.6 mg of RNA from *Escherichia coli* was added. To increase the proportion of the message fraction, log-phase cells were incubated in growth medium for 30 minutes in the presence of chloramphenicol (50 $\mu$g/ml). This has consistently resulted in a preparation of RNA extremely active in stimulating incorporation of amino acids. Again a control was run without added RNA. As shown, negligible amounts of histidine were incorporated and the same was true for valine.

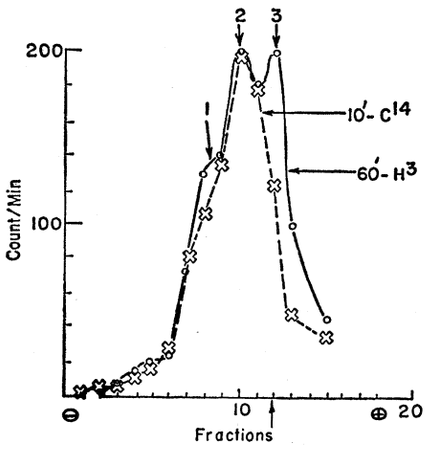


Fig. 4. Incorporation for different time intervals. Two-tenths milliliters of a reaction mixture (as described in Fig. 2) were incubated for 60 minutes at 35°C with $H^3$-labeled leucine, and 0.1 ml of another reaction mixture, incubated for 10 minutes at 35°C with $C^{14}$-labeled valine, were mixed. They were adjusted to 5*M* urea and .01*M* EDTA and loaded on the column at the point indicated by the arrow. Electrophoresis and subsequent treatment for counting is as described for Fig. 2.

dine). Again, there was a 5-to-6-minute lag in the incorporation of histidine and none with leucine. The lag cannot, therefore, be attributed to any difficulty in counting $H^3$ in the presence of excess $C^{14}$ (see also Fig. 3*B*).

The control preparations of Fig. 3*A* and 3*B*, to which RNA was not added, incorporated negligible quantities of either $C^{14}$-valine or $H^3$-histidine. There was the possibility that the system discriminated against the synthesis of histidine-containing proteins because of some defect which was restored within the 6-minute period. To check this eventuality, the experiment described in Fig. 3*B* was performed with the extract described in Fig. 3*A*. In this companion experiment, however, RNA message from *E. coli* was added. Since this is a heterogeneous population of messages, initial preferential synthesis of a given protein type would not be expected. Figure 3*B* shows no difference in entry times of histidine and valine. The difference observed in Fig. 3*A* must, therefore, be ascribed to the viral RNA.

It would be expected from Fig. 3*A* that early samples would be characterized by a predominance of the protein that does not contain histidine (peak 2). This expectation is realized as shown from the results in Fig. 4. Here an incorporation with $C^{14}$-valine was terminated at 10 minutes and mixed with another sample allowed to incorporate $H^3$-leucine for 60 minutes. The $H^3$ profile exhibits the presence of all three components. The $C^{14}$ profile shows a strong peak 2, traces of peak 1, and virtually no evidence of peak 3.

The results described in Figs. 3*A* and Fig. 4 are inconsistent with any random translation of the cistrons in the viral RNA. Nor are they in agreement with an ordered translation with equal frequency for all cistrons. The data are most readily interpreted if the translating device performs its function sequentially and, in so doing, makes significantly more product from the coat-protein cistron than from the others. The fact that the appearance of the histidine-containing proteins is delayed suggests that, of the three corresponding to the major protein components, the coat-protein cistron is the first to be translated. The data do not eliminate the possibility that a cistron coding for a minor protein component may precede the coat-protein cistron.

Thus, it is indeed possible to exhibit evidence in a cell-free system for a type of translational regulation which is predictable from a numerical analysis of what occurs in the intact cell. In a polycistronic message it determines the temporal order and the number of times a given cistron is used as a protein program. The experimental detection of translational regulation was made easier by the existence of polycistronic messages. It is clear, however, that the control of translation frequency can also be exerted on messages containing only a single cistron.

It seems likely that this type of control is not confined to the RNA viruses but may also regulate the translation of normal genetic messages. From the sizes observed, polycistronic messages are very likely to be found both in bacterial (*3–5*) and animal cells (*6*). Translational control would operate in addition to the transcriptional devices (*1, 2*). A degree of flexibility is provided that might well have selective advantages. Thus, even if two loci generated messages in equal numbers, the corresponding proteins might be synthesized with very disparate frequencies.

Various mechanisms can be proposed to account for the translational control devices detected. Although not the only one possible, the simplest way to explain the temporal order is to equate it to a corresponding linear sequential arrangement of the cistrons being translated. The frequency control might involve feed-back inhibition by product or some interaction between a component of the translating mechanism and a special sequence built into the message. This sequence may, in fact, be related to the intercistronic genetic punctuation.

The results show that a further analysis of the molecular basis of translational regulation may now be attainable in the comparative simplicity of a cell-free system (*25*).

*Note added in proof*: Since this manuscript was submitted a number of investigators independently reported similar conclusions concerning polycistronic messages at the 1963 Cold Spring Harbor Symposium. These included B. Ames and R. Martin, B. Guttman and A. Novick, A. Rich, I. Zabin, and N. Zinder.

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