

Interrelations between Nucleic Acid and Protein Biosynthesis in Microorganisms

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INTRODUCTION

Brachet (1) and Caspersson and Brandt (2) first drew attention to the possible participation of nucleic acids in protein synthesis. It has been proposed that the nucleic acids serve as templates for the formation of proteins and that they determine the sequence of amino acids in the protein molecule, and such a role has been supported on statistical grounds (3). Such a role for ribonucleic acid (RNA) has been experimentally demonstrated in the case of plant viruses (4, 5).

A more complex metabolic relationship between nucleic acid and protein formation has been suggested by recent investigations, indicating both an influence of nucleic acid components on the synthesis of protein and an effect of amino acids on nucleic acid formation. Thus, Pardee (6) has demonstrated an absolute uracil requirement for adaptive enzyme formation. He explained this requirement by postulating an obligatory coupling of RNA and protein synthesis; the RNA, once formed, being inactive. Gale and Folkes (7) have demonstrated a stimulation of protein synthesis by added RNA, which could, however, be replaced by RNA breakdown products. In the system of Webster and Johnson (8) protein synthesis, as measured by incorporation of labeled amino acids, required all the nucleosides or 5'-nucleotides occurring in RNA. No such clear-cut requirements exist in the amino acid incorporating microsomal systems studied by Zamecnik *et al.* (9) and by Borsook (10). As for the effect of amino acids on RNA, Gale and Folkes (7) have demonstrated a definite stimulation of nucleic acid formation by these compounds under

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conditions where protein synthesis does not occur, in the presence of chloramphenicol. However, these authors explained their results by a blocking of dissociation of nucleoproteins by chloramphenicol, the added amino acids allowing for the formation of more protein, thus permitting more associated RNA to be formed.

We have attempted to study further the role of amino acids in the biosynthesis of RNA, and the coupling between protein and nucleic acid synthesis. The existence of amino acid-requiring microorganisms, the demonstrated absence of turnover of RNA and protein in bacteria (11, 12), and the possibility of measuring net RNA and protein synthesis rather than incorporation of label, make them favorable objects for this investigation. In addition, the property of chloramphenicol to block protein formation without inhibiting nucleic acid synthesis in sensitive bacteria makes possible the direct study in intact cells of the amino acid requirement for nucleic acid biosynthesis.

After the work reported here was completed, a paper by Pardee and Prestidge (13) appeared, in which most of our results have been anticipated. We wish to report briefly our own results which were in part obtained using different organisms and their mutants, but which lead to essentially the same conclusions as those arrived at by these authors.

EXPERIMENTAL

Materials

The yeast used was a lysine-deficient mutant of *Saccharomyces cerevisiae* (No. 1496a) obtained from Dr. Carl Lindgren. The tryptophanless mutant of *Escherichia coli* (No. 567) was obtained from Dr. Francis J. Ryan, and the histidineless mutant of *Aerobacter aerogenes* (No. H-50) from Dr. Boris Magasanik. DL-Tryptan was generously supplied by Dr. H. R. Snyder. L-Lysine hydrochloride and L-tryptophan were commercial preparations. Chloramphenicol was a commercial preparation recrystallized from water.

Analytical Methods

Yeast protein was determined as nitrogen by a micromodification of the Nessler reaction (14); the nitrogen contributed by nucleic acid was subtracted in calculating the protein nitrogen values. Bacterial protein was determined by a modification of the biuret method (15). Deoxyribonucleic acid (DNA) was obtained by the Dische reaction modified as follows: the color was developed by leaving the reaction mixture at room temperature overnight instead of boiling it for 10 min.; the color intensity was increased about three-fold by this procedure.³ RNA and

³ Burton (16) has described a similar modification of the Dische reaction. However, using redistilled acetic acid, he finds it necessary to add acetaldehyde to the reaction mixture.

acid-soluble nucleotides were determined by their absorption at 260 $m\mu$ in phosphate buffer pH 7.0, using an average molar extinction of 11,300 per nucleotide (computed from a theoretical tetranucleotide). In the case of bacteria, the contribution of DNA to the total absorption, E_{260} of 9000/mole nucleotide/l., was subtracted in order to obtain the RNA values. No subtraction was made for the yeast RNA values, since the amount of DNA in yeast is insignificant in relation to the RNA content. The DNA extinction was obtained from a sample of *Aerobacter aerogenes* DNA treated with 0.1 *N* KOH at 30° for 24 hr. Phosphorus was determined by the method of King (17).

Yeast and Bacterial Cultures

Yeast was grown on a natural medium (1% bacto-peptone, 1% yeast extract, 2% glucose, tap water), washed in water, and kept in the cold for no more than a week before use. The basic synthetic growth medium used in the experiments was, expressed in g./l., $(\text{NH}_4)_2\text{SO}_4$ 0.7, glucose 40, KH_2PO_4 13.6, MgSO_4 0.012, K_2SO_4 0.25, succinic acid 1.0, trace elements and vitamins (expressed in $\mu\text{g./l.}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 300, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 200, $\text{B}(\text{OH})_3$ 5, $(\text{NH}_4)_2\text{MoO}_4$ 50, MnSO_4 50, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ 200, biotin 2.5, pantothenic acid 400, inositol 2000, nicotinic acid 400, *p*-aminobenzoic acid 200, pyridoxal 400, thiamine 400); lysine 20 mg./l. Phosphate starvation was carried out for 18 hr. in basic medium minus phosphate, the cells were washed in water, then suspended in the complete synthetic medium containing varying amounts of lysine.

Escherichia coli was grown on glucose 0.2%, Na_2HPO_4 0.2%, KH_2PO_4 0.2%, $(\text{NH}_4)_2\text{SO}_4$ 0.2%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.02%, CaCl_2 0.001%, tryptophan 20 mg./l. The medium for *Aerobacter aerogenes* (18) was glycerol 0.2%, Na_2HPO_4 0.54%, KH_2PO_4 1.26%, $(\text{NH}_4)_2\text{SO}_4$ 0.2%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.02%, CaCl_2 0.001%, histidine 500 mg./l. Eighteen-hour-old cultures were suspended for 1½ hr. in fresh medium for "rejuvenation," collected, washed in medium lacking amino acid, then incubated in the same medium for 1½ hr. to insure amino acid depletion. Enough chloramphenicol was added to portions of the "starved" cultures to make a final concentration of 100 $\mu\text{g./ml.}$ When required, the appropriate amino acid was added ½-1 hr. after the addition of chloramphenicol to allow enough time for the latter compound to take action (19). All incubations were carried out at 30° on a shaker.

Preparation of Samples

In the case of yeast, 50-ml. samples were collected, extracted with 5% trichloroacetic acid in the cold for 1 hr., washed with ether and alcohol, and treated with 1 *N* KOH at 30° for 20 hr. Aliquots were then withdrawn for protein determination, and the rest of the suspension brought to pH 4.5 with HCl and acetate buffer. The RNA ultraviolet spectrum of the resulting supernatant showed little contamination by protein.

The bacterial samples (usually 15 ml.) were subjected to the same treatment as yeast up to the stage of alkaline hydrolysis, which was carried out with 0.1 *N* KOH. The KOH hydrolyzate was used as such for protein, total nucleic acid, and DNA determinations; precipitation of the protein before nucleic acid determination was not necessary, since the distortion of the nucleic acid ultraviolet spectrum was insignificant.

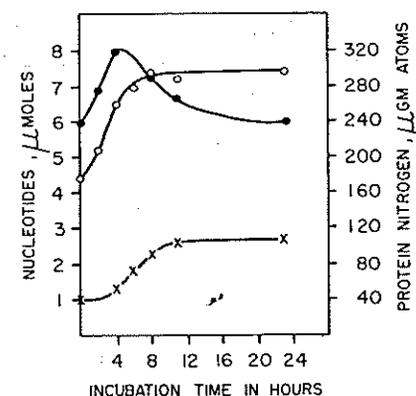


FIG. 1. Nucleic acid and protein changes during phosphate starvation. ○, protein nitrogen; ●, RNA; ×, acid-soluble nucleotides.

RESULTS

Wiame (20) has shown that by growing baker's yeast in a medium devoid of phosphate, the RNA content is much reduced. If phosphate is then added, a rapid synthesis of RNA occurs.

Using a lysine-deficient mutant strain, we have examined whether RNA synthesis in previously phosphate-depleted yeast is dependent on the presence of added lysine.

The course of events during a 24-hr. starvation period is shown in Fig. 1. For the first 4 hr. both RNA and protein increase, the quantity of RNA then begins to decrease, and at 24 hr. reaches a value slightly below that at the start of the starvation period. RNA begins to decrease when protein synthesis ceases. The acid-soluble nucleotides of the cells show an almost equivalent rise as the RNA falls.

On washing and transferring such starved yeast to a growth medium complete in all respects and containing varying amounts of lysine, the increase of RNA and of course protein is dependent on the amount of lysine added (Fig. 2). Without lysine there is no change in either RNA or protein. The amount of RNA and protein finally formed in this and other experiments is directly proportional to the amount of lysine added (Fig. 3). A feature of these results is the abrupt stoppage of RNA synthesis as opposed to the more gradual termination of protein formation as lysine is being exhausted.

Examination of Fig. 3 shows that 1 μ mole of lysine brings about the

formation of approximately 18 μg . atoms of protein nitrogen and 1 μmole of RNA nucleotide. Assuming an average value of 1.2 nitrogen atoms per amino acid residue in yeast protein, 15 moles of amino acid residues polymerize per mole of nucleotide assembled into RNA.

As Wiame has shown, transfer of phosphate-starved yeast to a phosphate-containing medium results in a very large and rapid uptake of phosphate into the cells, mainly into metaphosphate fraction. As we show, phosphate uptake into RNA depends on the presence of lysine. Not all phosphate uptake is, however, lysine dependent, since even in

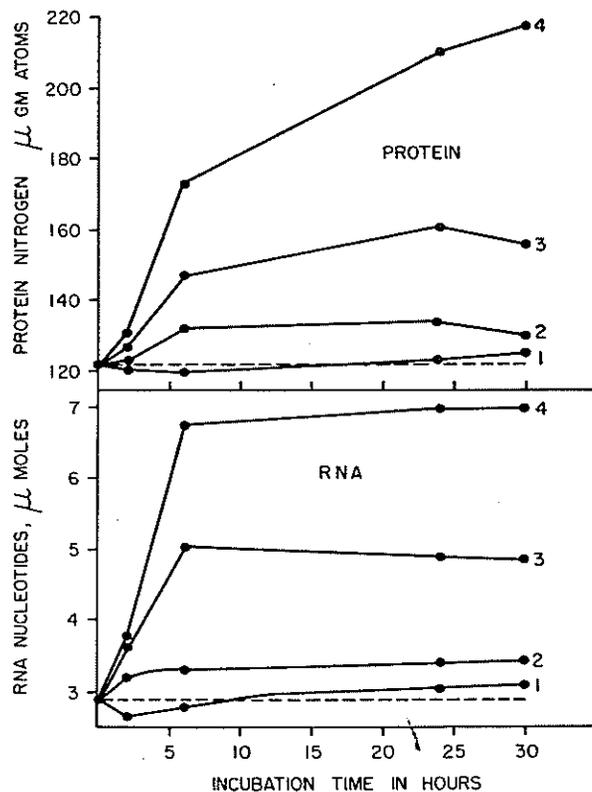


FIG. 2. Increases in protein and ribonucleic acid upon incubation with limiting amounts of lysine. Lysine in medium, in $\mu\text{moles}/50\text{ ml.}$: (1) 0; (2) 0.54; (3) 2.16; (4) 5.40.

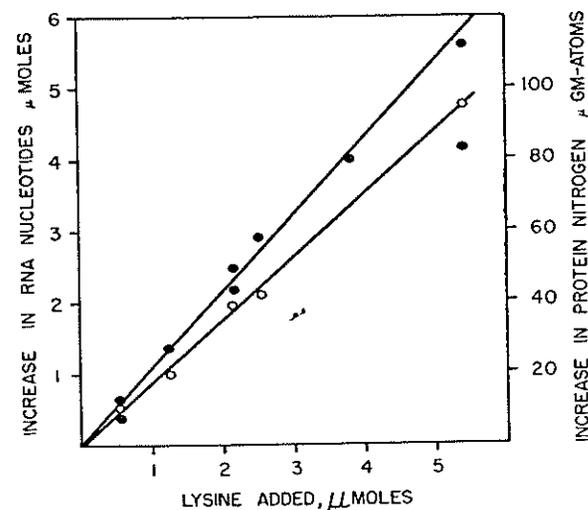


FIG. 3. Relation between amounts of lysine added and quantity of protein and nucleic acid formed.

●, ribonucleic acid; ○, protein nitrogen.

the absence of lysine, phosphate-starved cells take up much phosphate, although less than in its presence (Table I). Thus the lysine requirement is, to a certain extent at least, specific for RNA formation but not for phosphate uptake into other fractions.

TABLE I
Effect of Lysine on the Changes in RNA and Total Phosphate of Phosphate-Starved Yeast

	Dry weight mg.	Total phosphate		RNA (as nucleotides)	
		μmoles	$\mu\text{moles}/\text{mg.}$	μmoles	$\mu\text{moles}/\text{mg.}$
Phosphate starved ^a	100	19	0.19	5.0	0.050
Phosphate replenished ^b	100	59	0.59	4.5	0.045
Complete growth medium ^c					
Without lysine	93	100	1.08	5.5	0.065
With lysine	143	194	1.36	21.5	0.150

^a Incubated for 18 hr. in phosphate-free synthetic growth medium.

^b Phosphate-starved yeast incubated for 1.5 hr. in 0.1 M KH_2PO_4 and 2% glucose.

^c Phosphate-starved yeast incubated for 5 hr. in synthetic growth medium.

As has been found by Gale and Folkes (7) and by Wisseman *et al.* (19), in chloramphenicol-sensitive bacteria it is possible to suppress protein synthesis completely by chloramphenicol and yet permit RNA synthesis to continue. Gale and Folkes (7) claimed that RNA synthesis is actually stimulated by the antibiotic, while Wisseman *et al.* (19) find merely that the normal rate of RNA synthesis is unimpaired.

We have investigated the effects of chloramphenicol on RNA and protein synthesis, using amino acid-requiring mutants of *E. coli* and *Aerobacter aerogenes*. On addition of chloramphenicol to bacteria in the logarithmic phase of growth, we have confirmed the conclusions of Wisseman *et al.* (19). Protein synthesis is completely suppressed, while RNA and DNA syntheses continue unimpaired for at least 1 hr. at the same rate as they were proceeding at the moment of addition of the antibiotic. In this system there is neither stimulation nor inhibition of nucleic acid synthesis, only protein synthesis being suppressed.

When a logarithmic phase culture of a tryptophan-requiring mutant of *E. coli* is washed and suspended in a medium otherwise complete but lacking tryptophan, there is no increase in either protein or RNA. This is in conformity with the results obtained with yeast. If chloramphenicol is added to such a static system, there is a resumption of RNA synthesis, with no increase in protein (Fig. 4). Chloramphenicol, therefore, in some manner "uncouples" the synthesis of RNA from that of protein.

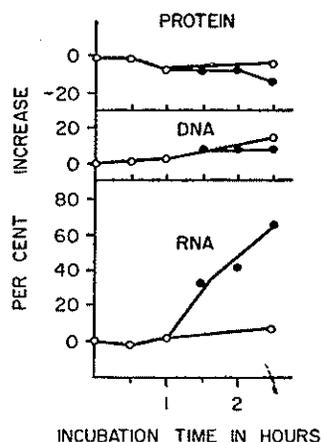


FIG. 4. Effect of chloramphenicol on tryptophan-deficient *E. coli*. ○, in tryptophan-free medium; ●, chloramphenicol added (after 1 hr.).

We have attempted to determine whether RNA synthesis is normally dependent on protein synthesis, or whether it is the lack of free amino acid which separately and independently prevents the synthesis of protein and RNA. Since in the presence of chloramphenicol no protein synthesis can take place, any effect of amino acids or amino acid analogs on chloramphenicol-activated RNA synthesis cannot be via any effect on protein synthesis but must act in some other manner.

At first sight, the activation of RNA synthesis by chloramphenicol in tryptophan-starved *E. coli* would appear to indicate that there is no amino acid requirement for RNA synthesis. If, however, chloramphenicol acts to suppress a residual synthesis of protein too small to detect by our analytical methods, addition of the antibiotic might cause an accumulation of small amounts of tryptophan in the free amino acid pool, either through protein breakdown or because the genetic block to tryptophan synthesis might not be quite complete. If amino acids are needed in catalytic amounts for RNA synthesis, chloramphenicol could "uncouple" RNA synthesis from that of protein by making such quantities of free amino acid available.

We have therefore attempted to demonstrate an inhibition of chloramphenicol-activated RNA synthesis by tryptazan, reported by Halvorsen, Spiegelman, and Hinman (21) to be an effective inhibitor of adaptive enzyme formation in yeast. We found, however, that using our tryptophan-requiring *E. coli* mutant, tryptazan is not an inhibitor of protein synthesis but instead permits fast although ultimately limited growth if supplied to the organism instead of tryptophan.

It has, however, proved possible to demonstrate an amino acid dependence of chloramphenicol-activated RNA synthesis using a histidine-requiring mutant of *Aerobacter aerogenes* originally studied by Magasanik and Bowser (22). This mutant has the peculiarity that it not only lacks the ability to synthesize histidine, but also has a powerful adaptive histidinase, breaking down histidine rapidly. It might, therefore, be expected that on histidine starvation the free histidine level of its amino acid pool might become significantly depleted.

When cells of this mutant are harvested during the logarithmic growth phase, washed, and starved for histidine, the addition of chloramphenicol does not always suffice to initiate the expected formation of RNA. Addition of histidine after that of chloramphenicol greatly increases the rate of RNA formation (Fig. 5). However, the histidine effect is erratic, and in spite of numerous attempts we have not been able to determine

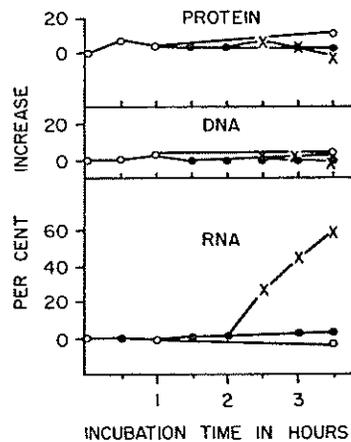


Fig. 5. Effect of chloramphenicol on the histidine-deficient mutant of *Aerobacter aerogenes*.

○, in histidine-free medium; ●, chloramphenicol added (after 1 hr.); ×, histidine added (300 mg./l., after 2 hr.).

the exact conditions under which it can be regularly produced. If the chloramphenicol-induced RNA synthesis is low, addition of histidine never fails to increase the rate of RNA formation. Frequently, however, the rate of RNA formation on addition of chloramphenicol alone is already maximal, and subsequent addition of histidine fails to increase it further. We attribute this rather unexpected variability to the difficulty of completely depleting the cells of histidine. Ushiba and Magasanik (18) have indeed shown that this same organism has some capacity for adaptive enzyme formation in the absence of exogenous histidine, pointing to the existence of some store of available histidine even during histidine starvation.

The effect of histidine on RNA synthesis cannot be due to the formation of a histidine-containing enzyme required for RNA formation, since the presence of chloramphenicol prevents protein formation. It is also improbable that histidine contributes material to RNA, since the carbon atoms of histidine in *Aerobacter aerogenes* do not appear in nucleic acid purines.⁴ At least four other amino acids have been shown by Pardee and Prestidge (13) to stimulate RNA synthesis in the presence of chloramphenicol.

⁴ B. Magasanik, personal communication.

DISCUSSION

From the results obtained with yeast and amino acid-deprived bacteria, it is clear that normally the synthesis of RNA does not occur unless the synthesis of protein is taking place at the same time. Our results with yeast are similar in several respects to those obtained by Schmidt *et al.* (23) who found that phosphate-starved yeast, returned to a phosphate-containing medium, failed to form RNA unless sulfate was present, and that the synthesis of RNA was inhibited by the amino acid analog ethionine.

The major problem is the nature of the normal "coupling." Several explanations can be envisioned and have, in fact, been proposed:

(a) Nucleic acid and protein are formed simultaneously, one being the by-product of the process of formation of the other (6). This hypothesis, attractive when first proposed, suffers from the fact that there is no simple stoichiometry between the amounts of RNA and protein formed, protein formation being greatly in excess.

(b) RNA can only be formed when the appropriate protein is available with which the newly synthesized RNA can form a nucleoprotein complex (7). This hypothesis is virtually disproved by the results obtained with chloramphenicol, where rapid RNA synthesis is possible in the complete absence of protein formation.

(c) The formation of RNA and protein is coupled because both are formed from common precursors. We believe this hypothesis adequately explains the facts known to date.

The precursors would be compounds involving both an amino acid and a nucleotide. A series of such compounds could polymerize to form either protein or nucleic acid, depending on what kind of template it is aligned on. In this manner the nucleotides (or some derivative thereof) would be released during protein formation and act as catalysts, and the same would apply to the amino acids during the formation of RNA.⁵ This hypothesis, of course provisional, has the merit of offering an explanation

⁵ Hoagland *et al.* (24) and De Moss *et al.* (25) have presented evidence for the occurrence of acyl adenylate derivatives of amino acids as intermediates in the synthesis of proteins. The compounds suggested in this paper could possibly have a similar structure, but, because they must involve all four RNA nucleotides in order to lead to nucleic acid as well as protein synthesis, they would have to be formed by a system different from the one involved in the acyl adenylate derivatives. This does not exclude the possibility that the initial activation of amino acids is via the mechanism studied by Hoagland and De Moss.

for the following facts and suggesting further experiments:

1. In amino acid depletion, RNA synthesis stops because a functioning protein-forming system depletes the amino acid pool.

2. On addition of chloramphenicol to a static (amino acid-depleted) system, formation of protein is completely blocked. This allows amino acids to accumulate, and since only catalytic amounts are required, the synthesis of RNA can proceed. This explains the "uncoupling" action of chloramphenicol in the tryptophan-requiring mutant of *E. coli* in terms of its known mode of action, the blocking of protein synthesis, without invoking another mode of action. Of numerous reactions studied, only protein synthesis has so far been found to be significantly inhibited by the antibiotic (26, 27).

3. If the amino acid pool could be kept depleted, chloramphenicol alone should fail to initiate the formation of RNA. This is obviously difficult to arrange, since the addition of chloramphenicol blocks the protein-forming mechanism responsible for keeping the amino acid pool depleted.

Our experiments using a histidine-requiring mutant of *Aerobacter aerogenes* suggest that such a situation is possible. A similar effect has been described by Gale and Folkes (7), who found that in their system the rate of RNA synthesis in the presence of chloramphenicol is stimulated by the addition of amino acids. Results essentially similar to ours, using four other amino acid-requiring mutants of *E. coli*, have been published by Pardee and Prestidge (13), and our conclusions are generally similar.

If it is indeed correct that the precursors of RNA are nucleotide-amino acid types of compounds, then it is probable that the polymerizing enzyme described by Grunberg-Manago and Ochoa (28) is not directly involved in natural RNA synthesis, since the substrates for that enzyme are nucleoside diphosphates.

A further consequence of this hypothesis should be pointed out. If intermediates of the type we postulate are RNA precursors, then a minimum of 20 such precursors presumably exist. Since omission of a single amino acid stops RNA synthesis, this would imply that the RNA-forming system, in order to form a polymer end product of only four different elements, must nevertheless distinguish in the process of formation at least 20. The informational content of the RNA-forming system would therefore be greatly redundant with respect to the end product.

SUMMARY

The synthesis of ribonucleic acid by amino acid-requiring mutant strains of yeast and bacteria is dependent on the presence of the corresponding amino acid. In histidineless *Aerobacter aerogenes*, histidine is required even under conditions of no protein synthesis. A hypothesis to account for these facts is discussed.

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