

## Origin of Chloramphenicol Particle Protein

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These experiments show that the majority of the protein found associated with the RNA synthesized in the presence of chloramphenicol is nonribosomal protein existing in the cells at the time of addition of the drug and hence that chloramphenicol particles are not incomplete ribosomes. The experiments are the following:

(a) The pool of free ribosomal protein as measured kinetically in normally growing cells is too small to provide the protein found on chloramphenicol particles, showing the chloramphenicol particle protein cannot derive solely from a pool of free ribosomal protein.

(b) The specific activity of chloramphenicol particle protein is independent of the time between a pulse labeling of cellular protein and the addition of the drug, showing that the protein found on chloramphenicol particles is not normally incorporated into some cellular structure from which it is unavailable to form the particles.

(c) The amount of protein which is synthesized in 90 minutes of chloramphenicol treatment is less than 3% of the protein found on chloramphenicol particles, showing that nearly all of the protein of the particles must have been synthesized before the drug was added.

(d) The specific activity of chloramphenicol particle protein parallels the specific activity of soluble protein and not ribosomal protein when the particles are prepared in cultures with different relative specific activities of ribosomal and non-ribosomal protein.

(e) Upon removal of chloramphenicol, the RNA of the chloramphenicol particles is matured to ribosomes while the protein previously sedimenting with this RNA is not found on mature ribosomes.

### 1. Introduction

The addition of chloramphenicol to exponentially growing *Escherichia coli* cultures can reduce the rate of protein synthesis more than a hundredfold; but initially, RNA synthesis is scarcely affected by the drug, and the RNA content per cell approximately doubles during 90 minutes of CM† treatment. Much of the RNA synthesized in the presence of the drug is found associated with protein in particles that sediment at 18 to 25 s and are called chloramphenicol particles. RNA extracted from these particles sediments at 16 s and 23 s, like the RNA extracted from mature ribosomes, and the current assumption is that CM particles are incomplete ribosomes and that the protein associated with them is drawn from a pool of free ribosomal protein existing in the cell at the time of addition of chloramphenicol (Hosokawa & Nomura,

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‡ Abbreviation used: CM, chloramphenicol.

1965). This hypothesis predicts that a sizeable pool of free ribosomal protein exists in normally growing cells. However, kinetic measurements indicate that the pool of free ribosomal protein is very small (Schleif, 1967b). The experiments reported here are interpreted as showing that the majority of the protein of CM particles is not ribosomal, but soluble protein present in the cells at the time of addition of the drug, and hence that CM particles most probably are not immature ribosomes.

These experiments depended on the quantitative separation of CM particles and ribosomes from cell wall and soluble protein; this was achieved by centrifuging through a high-density  $D_2O$ -sucrose gradient.

## 2. Materials and Methods

### (a) Cell growth and preparation of extracts

*E. coli* B/r was grown on a minimal mineral salts medium with glucose or succinate present at 0.2%—giving doubling times of 50 and 100 min, respectively. In an enriched minimal medium, the doubling time was 35 min. After adding supplements, glucose was 0.2%; adenine, guanine, cytosine and uracil were each 40  $\mu\text{g}/\text{ml}$ .; leucine was 20  $\mu\text{g}/\text{ml}$ .; and the concentrations of 18 other amino acids were in proportion to leucine as they occur in *E. coli* protein (Roberts, Abelson, Cowie, Bolton & Britten, 1963). Proline was omitted to allow its use as a radioactive label. Cells were grown exponentially for at least six doublings before an experiment.

For the standard 90-min chloramphenicol treatment of cells, freshly dissolved chloramphenicol was added to give a concentration of 100  $\mu\text{g}/\text{ml}$ . Media were changed by filtration through 0.45  $\mu$  Millipore filters that had been washed with boiling water to remove plasticizers. In preparation of cell extracts, 100 ml. of culture were concentrated to 2 ml., washed 3 times in TM buffer (0.005 M-Tris-HCl (pH 7.2), 0.0001 M-magnesium acetate), and concentrated to 0.3 ml. before adding 10  $\mu\text{g}$  lysozyme, 1  $\mu\text{g}$  DNase, freezing in dry ice mixed with ethanol and thawing 5 min at 37°C for a total of four cycles. This treatment disrupted 70 to 90% of the cells and apparently does not degrade the CM particles. Unbroken cells and large fragments of cell wall were removed by centrifugation at 10,000 g for 15 min. Cells not treated with chloramphenicol were disrupted by sonication, giving greater than 99.9% disruption (without the CM treatment, strain B/r is not very sensitive to lysis by the freeze-thaw procedure), and the resulting extract was centrifuged unfractionated through the  $D_2O$ -sucrose gradients. Measurements of the ratio of radioactive proline in ribosomes to total cellular protein made on cultures lysed by sonication and by lysozyme-freeze-thaw show that the fragments of cell wall from cells lysed by freezing and thawing which are pelleted by centrifugation at 10,000 g for 15 min contain approximately 20% of the cellular protein.

### (b) Purification of ribosomes and chloramphenicol particles

Cell extracts were centrifuged through 5 to 20% sucrose gradients or  $D_2O$ -sucrose solutions with density varying linearly from  $\rho = 1.20$  to 1.30. The ion concentrations in all gradients were the same as in TM buffer. The increased density of the  $D_2O$  gradients prevents cell wall or cell membrane from sedimenting with ribosomes or CM particles, but the higher viscosity and density increase the time required to sediment 50 s ribosomes 3 cm in the Spinco SW 39 rotor from 2 hr at 4°C and 110,000 g to 20 hr at 20°C and 107,000 g.

Drops from the centrifuge tube were collected directly in scintillation vials and counted. The masses of CM particles and ribosomes were estimated in analytical centrifugation by measuring areas under the peaks produced by schlieren optics in velocity runs (Nomura & Watson, 1959). Compared to the ribosomes, the CM particles were not more stable when the RNase I minus strain D10 (Gesteland, 1966) was used instead of strain B/r. Therefore, for comparison with previous studies, strain B/r was used for all measurements. For additional details see Schleif (1967b).

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(a) Cells contain insufficient  
(i) Amount of protein

To measure the amount of protein, cells were treated for 90 min with chloramphenicol and centrifuged. Ribosomes were separated from the presence of chloramphenicol particles into ribosomes and sedimented. The amount of protein before be estimated by counting the radioactivity of ribosomes in the same extract. The amount of protein was more than half of the total protein. CM particles seem to contain more protein (Watson, 1959); hence the amount of protein in the extract is about 25% of the total protein.

(ii) Amount of free ribosomes

A previous measurement here showed that there is a significant appearance of radioactive

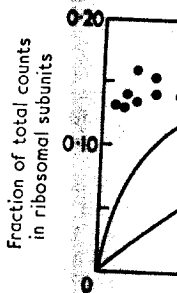


Fig. 1. Measured kinetics of a chase beginning 1 min after the expected kinetics of label entry. The amount of protein already

one-minute pulse labeling a cell if the pool contained either free mature ribosomes (Schleif, 1967b) or a pool (or if each of the free protein before being incorporated into ribosomes certainly contain less than 8% of the protein in mature

### 3. Results and Conclusions

(a) Cells contain insufficient quantities of free ribosomal proteins to supply CM particles

(i) Amount of protein in CM particles

To measure the amount of protein in the CM particles, extracts from cells which had been treated for 90 minutes with chloramphenicol were analyzed in the analytical centrifuge. Ribosomes synthesized during normal growth are not degraded in the presence of chloramphenicol, since all the radioactive uracil, or proline, incorporated into ribosomes sediments in ribosomal subunits, at 30 s and 50 s, even after treatment with 100  $\mu\text{g}$  of CM/ml. for 90 minutes. The total protein on CM particles can therefore be estimated by comparing the amount of CM particles to the amount of ribosomes in the same extract. It was found that the material sedimenting at 18 to 25 s was more than half of the mass of ribosomes in the cell extract. Per unit of RNA, the CM particles seem to contain about half the protein found in ribosomes (Nomura & Watson, 1959); hence the quantity of protein associated with the CM particles in the extract is about 25% of the total ribosomal protein.

(ii) Amount of free ribosomal protein in growing cells

A previous measurement on cells growing exponentially under the conditions used here showed that there is a very small pool of free ribosomal protein. The kinetics of appearance of radioactive proline in the protein of mature ribosomes following a

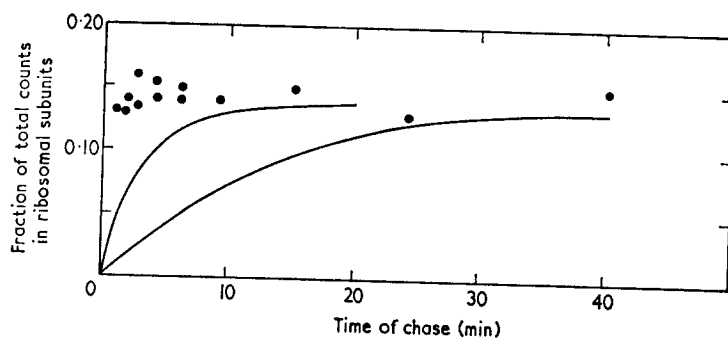


Fig. 1. Measured kinetics of appearance of label in mature ribosomal subunits as a function of time of a chase beginning 1 min after addition of a pulse label ( $\bullet$ ). The two curves show the expected kinetics of label entry if the pool contained 4% (upper curve) and 20% (lower curve) of the amount of protein already present in mature ribosomes.

one-minute pulse labeling are shown in Figure 1. Also shown are the expected kinetics if the pool contained either 4% or 20% of the amount of protein already present in mature ribosomes (Schleif, 1967a). Thus it is clear that if all ribosomal proteins pass through a pool (or if each of the 35+ proteins were to pass through its own pool) of free protein before being incorporated into a ribosome, then the pool (or pools) certainly contain less than 4% *in toto* of the amount of protein already present on mature ribosomes. Conceivably, some of the proteins pass through very small pools while the rest pass through pools of appreciable size. An analysis of this case indicates that the kinetics shown in Figure 1 are incompatible with a pool containing more than 8% of the protein in mature ribosomes (Schleif, 1967a).

(b) *Non-transient pool of CM particle protein*

If the protein of the CM particles were drawn from a pool which normally supplies protein to *stable* structures, including the ribosomes, then the greater the time between pulse labeling of cellular protein and the addition of chloramphenicol, the lower would be the specific activity of the CM particles. In fact, the specific activity of CM particles was found to be independent of the time between pulse labeling and addition of chloramphenicol, indicating that the protein labeled at a given time remains available indefinitely for incorporation into CM particles. The plan of this experiment is shown in Figure 2, where  $T$  was the variable. The RNA of the CM particles was labeled with [ $^3\text{H}$ ]uracil to assay the recovery of CM particles. The radioactivity of CM particle protein was accurately measured as the ratio of [ $^{14}\text{C}$ ]proline to [ $^3\text{H}$ ]uracil in purified particles, obtained by centrifuging cell extracts through 5 to 20% sucrose gradients (107,000 g, 3 hr, 20°C), taking out a CM particle fraction, as shown in Figure 3, and centrifuging this through linear  $\text{D}_2\text{O}$ -sucrose gradients for 24 hours. The recovery of radioactivity from each centrifugation was better than 75%.

The first centrifugation separated CM particles from 30 s subunits, and the second separated CM particles from other cell protein. The profile of the  $\text{D}_2\text{O}$  gradient is

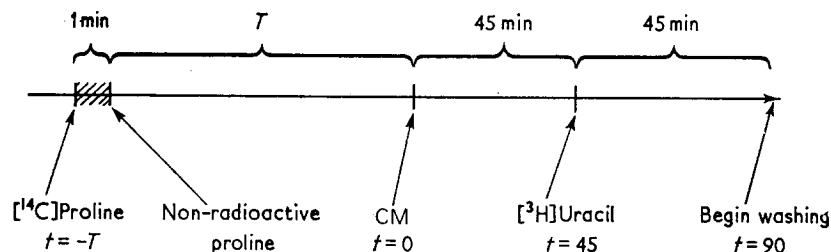


FIG. 2. Plan for kinetic measurement of pool size of CM particle protein.

To six identical 50-ml. cultures CM was added to make a concentration of 100  $\mu\text{g}/\text{ml}$ .; 45 min later 5  $\mu\text{C}$  of [ $^3\text{H}$ ]uracil was added in a quantity that would be completely incorporated in less than 5 min. At various times,  $T$ , before drug addition, 1  $\mu\text{C}$  of [ $^{14}\text{C}$ ]proline had been added in such a quantity that it was estimated would be just incorporated into the earliest labeled sample in 1 min.

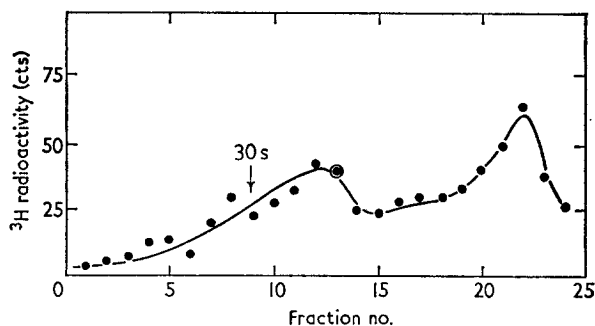


FIG. 3. A typical profile from a preparative gradient centrifugation.

0.2 ml. of the extract prepared from cells labeled 40 min before addition of CM was layered on a 4.5-ml. linear 5 to 20% sucrose gradient and spun at 107,000 g, 3 hr, 20°C. 0.01-ml. samples from each 3-drop fraction were counted. The graph shows the  $^3\text{H}$  profile (—●—●—) and the fraction (○) which was layered on the  $\text{D}_2\text{O}$  gradient.

presented in Figure 4. The specific activity of  $^{14}\text{C}$  to  $^3\text{H}$  across the CM particles decreases as the time between proline and the addition of chloramphenicol increases. This is due to the fact that the protein normally goes elsewhere and is not available for incomplete utilization of the pulse label.

This is more easily seen if the sample labeled with [ $^{14}\text{C}$ ]proline is taken up only a short time after it had been stopped. The specific activity measurement, when the CM particles are separated from the cells had incorporated

FIG. 4. Profile of [ $^{14}\text{C}$ ]proline and [ $^3\text{H}$ ]uracil in CM particles. Centrifugation was carried out on a  $\text{D}_2\text{O}$ -sucrose gradient. The light fraction is the fraction which would have been

FIG. 5. Specific activity of proline and addition of

[ $^3\text{H}$ ]uracil (cts  $\times 10^{-3}$ )

Spec. act. of CM particles ( $^{14}\text{C}/^3\text{H}$ )

presented in Figure 4, which shows the clean separation of CM particles from cell wall material. The specific activity of the particles was estimated from the constant ratio of  $^{14}\text{C}$  to  $^3\text{H}$  across the CM particle peak. Figure 5 shows that the specific activity of CM particles decreases with time between the end of a one-minute pulse labeling with proline and the addition of chloramphenicol much more slowly than could be accounted for by a pool of any reasonable size. This shows the protein of CM particles does not normally go elsewhere. Furthermore, the slope of the line in Figure 5 probably reflects a somewhat lower specific activity of the proteins in the early samples due to incomplete utilization of the added proline; cf. legend to Figure 2, which shows that the earlier the pulse the lower the cell density during incorporation.

This is more easily understood by the following hypothetical situation. Suppose the sample labeled with proline at the largest  $T$  (earliest sample before CM addition) had taken up only a small fraction of the radioactive proline before further incorporation had been stopped by the addition of excess non-radioactive proline. At the end of the measurement, when the proline counts are compared with the uracil counts in the CM particles, this sample will have a low ratio of proline activity to uracil activity, since the cells had incorporated less total proline but the same amount of uracil as other samples.

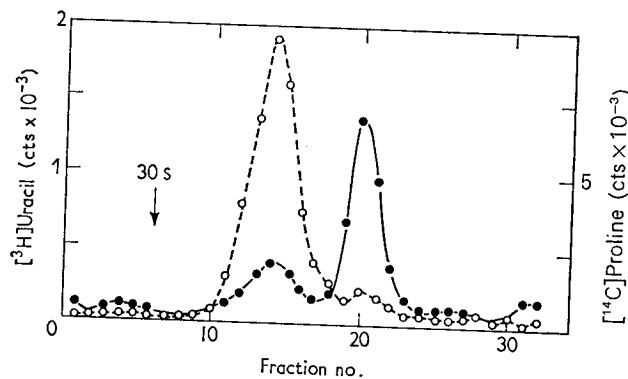


FIG. 4. Profile of [ $^3\text{H}$ ]uracil (—○—○—) and [ $^{14}\text{C}$ ]proline (—●—●—) from the fraction indicated (○) in Fig. 3.

Centrifugation was at 107,000 g, for 24 hr, at 20°C. The  $^3\text{H}$  peak shows the position of the CM particles, and the lighter peak at fraction 20 shows cell wall or membrane. The peak at fraction 14 could be removed by 20 min of treatment with RNase at 1  $\mu\text{g}/\text{ml}$ . before centrifugation. This fraction is not contaminated with significant amounts of 30 s ribosomal subunits, the presence of which would have skewed the  $^{14}\text{C}$  profile towards the faster sedimenting ribosomes (cf. Fig. 7).

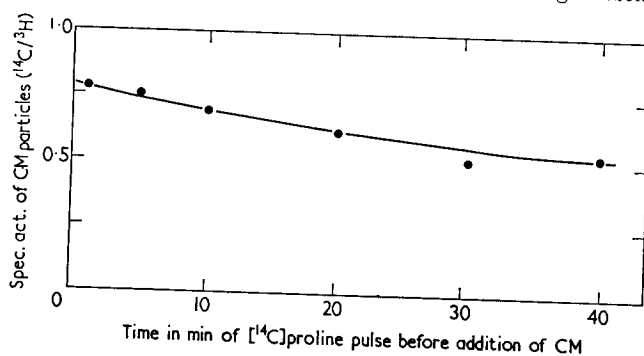


FIG. 5. Specific activity of CM particles as a function of the time between pulse labeling with proline and addition of CM.

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(c) *Origin of CM particle protein*

Since it has been shown that CM particle protein does not derive from a pool of free ribosomal protein, we are left with four *a priori* possibilities: (a) the CM particle protein could be ribosomal or non-ribosomal protein synthesized in the presence of chloramphenicol; (b) it could be ribosomal protein from ribosomes degraded in the presence of chloramphenicol; (c) it could be non-ribosomal protein existing in the cells at the time of addition of chloramphenicol; or (d) it could derive from a combination of these sources.

Suggestion (a) can be rejected, since addition of 100  $\mu\text{g}$  of chloramphenicol/ml. reduces the rate of protein synthesis under the conditions used by 200-fold, as measured by uptake of radioactive proline and leucine. At this rate less than 3% of the protein found on CM particles would be produced in 90 minutes, even if nothing but this type of protein were synthesized. In fact, one-third of the leakage synthesis is ribosomal protein. This was measured by adding chloramphenicol at time zero and the radioactive proline 10 minutes later. The proline and chloramphenicol were removed by separate filtrations at 50 and at 65 minutes, respectively, after which the culture was allowed to double in optical density before measuring the radioactivity in ribosomes and in total protein.

If all or most of the CM particle protein derived from ribosomes degraded in the presence of CM (suggestion (b)), then the specific activity of CM proteins in cells labeled with [ $^{14}\text{C}$ ]proline before the addition of chloramphenicol would be the same as the specific activity of ribosomal protein at the time of addition of the drug. The relative specific activities of ribosomal and soluble protein were varied over a ten-fold range in these experiments, and it was found that the specific activity of CM particle protein followed more closely that of the soluble protein.

The specific activity of the ribosomal protein was varied relative to that of the soluble proteins by labeling the cells during growth at different rates. The rate of synthesis of ribosomes relative to soluble protein is high in a medium supporting rapid growth. Thus short-time labeling with [ $^{14}\text{C}$ ]proline during rapid growth and subsequent balanced growth at a much lower rate give ribosomes the proteins of which have a higher specific activity than the soluble proteins (30% of label enters ribosomes). Conversely, labeling during a period when the relative rate of ribosome synthesis is almost zero—e.g. shortly after shift of cells from rapid to slow growth—and subsequent balanced growth at a high rate, give ribosomes with a relatively low specific activity (3% of label enters ribosomes). Using cells in balanced, exponential growth in glucose minimal medium, but having (i) 0.03 and (ii) 0.30 of the total incorporated radioactive material in the proteins of the mature ribosomes (achieved by the method outlined above, and shown explicitly in Fig. 6), it was found that the specific activity of the proteins of CM particles subsequently produced paralleled that of *soluble* protein and *not ribosomal* protein.

Figure 7 shows the radioactive profiles from the extracts of cells (i) and (ii) above. The [ $^{14}\text{C}$ ]uracil profiles indicate that the RNA synthesized after addition of CM is similar in the two frames. Not shown, but also similar, would be the profiles of ribosomes and soluble protein. In Figure 7 (i), it is seen that almost all the [ $^3\text{H}$ ]proline label has entered non-ribosomal protein, whereas in Figure 7 (ii) a large fraction of the [ $^3\text{H}$ ]proline is in the ribosomes.

Since the cultures represented by frames (i) and (ii) of Figure 7 were growing exponentially in identical media, and thus contained the same quantities per cell

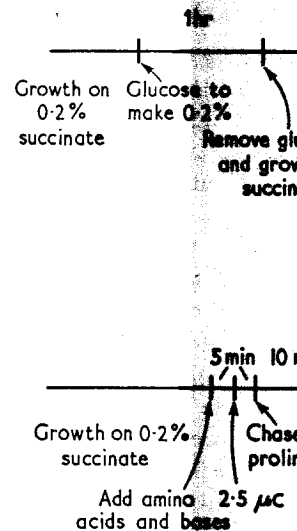


FIG. 6. Treatment of cells so that ribosomal protein, but will other

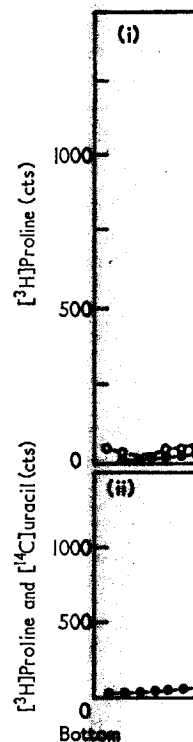


FIG. 7. (i) [ $^3\text{H}$ ]Proline (—) prepared from cells treated as for 21 hr. (ii) Same as (i), but extract

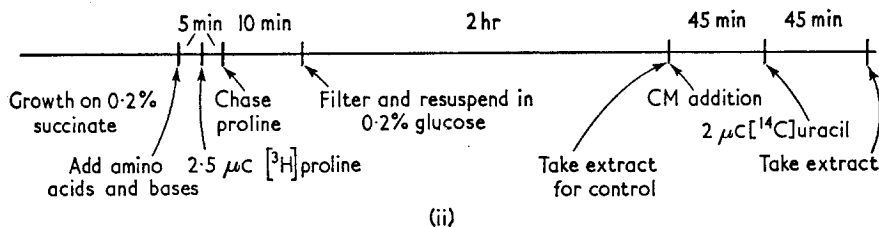
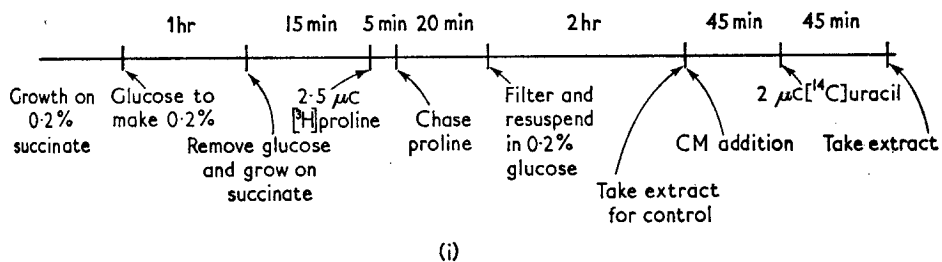


Fig. 6. Treatment of cells so that they will incorporate (i) 0.03, (ii) 0.30 of the proline label into ribosomal protein, but will otherwise be similar in ribosome content at the time of addition of CM.

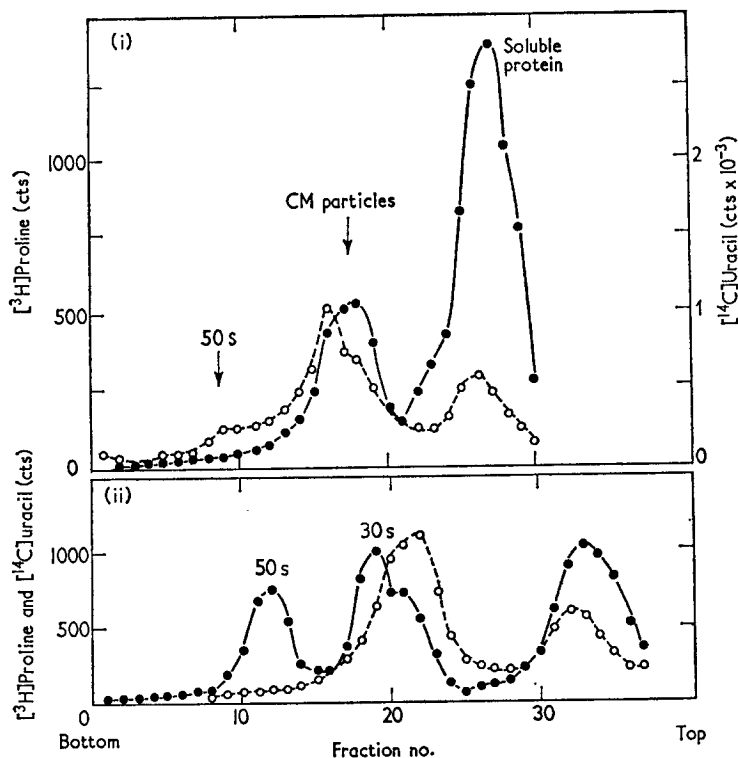


Fig. 7. (i)  $[^3\text{H}]$ Proline (—●—●—) and  $[^{14}\text{C}]$ uracil (---○---○---) profiles of the extract prepared from cells treated as in Fig. 6 (i) and centrifuged through  $\text{D}_2\text{O}$ -sucrose 107,000 g,  $20^\circ\text{C}$  for 21 hr.

(ii) Same as (i), but extract Fig. 6 (ii).

of ribosomal, as well as non-ribosomal, protein, the *specific activity* of non-ribosomal protein, relative to that of ribosomal protein, is higher in case (i) than in case (ii). Examining the CM particle regions, indicated by the [ $^{14}\text{C}$ ]uracil counts, it is seen that similar amounts of radioactive proline entered that region in the two cases, despite the fact that the specific activity of the ribosomal protein was about ten times less in case (i) than in case (ii).

This experiment was designed to show that CM particle protein originates largely from non-ribosomal protein. The method of demonstration used was to show that increasing the specific activity of ribosomal proteins synthesized before addition of CM would not significantly alter the amount of radioactive amino acids subsequently found in CM particles. Thus it appears from Figure 7 (i) and (ii) that the 10-fold increase in ribosomal protein specific activity was not matched by a corresponding 10-fold increase in CM particle specific activity. There are several limitations to absolute quantitation of the data. The first is apparent in Figure 7 (i), where it can be seen that there was not complete correspondence between the uracil activity marking the location of CM particles and the protein of CM particles. This most probably originates from the heterogeneity of the CM particles, which will be discussed later. It is also possible that during the extended centrifugation of 21 hours at 20°C some of the CM particle protein was released from the RNA. Another fact which prevents absolute quantitation is the presence of proteins which are neither ribosomal nor chloramphenicol particles in origin and which would decrease the magnitude of the difference in chloramphenicol particle activities seen in Figure 7 (i) and (ii). It was necessary to use the high-density  $\text{D}_2\text{O}$  gradients to overcome this difficulty, for with the usual sucrose gradients the amount of protein, predominantly cell wall, was too high to resolve adequately ribosome peaks (Schleif, 1967*a,b*). Even with these precautions, the possibility of an impurity in the CM particle region of up to 20% of the amount of chloramphenicol particle protein cannot be excluded.

A direct proof that CM particle protein is not ribosomal protein would be to show that, upon removal of chloramphenicol and subsequent disappearance of the CM particles, their protein returns to the class of soluble protein rather than entering mature ribosomes (suggested by Gunther Stent). Figure 7 shows that the radioactivity in the CM particle proteins is most easily measured when the specific activity of ribosomal protein is low compared to soluble protein. For this reason cells were grown and labeled as in Figure 6 (i). The sample taken just before addition of chloramphenicol to the remainder had about 0.03 of the label in ribosomes. The sample taken for analysis after 90 minutes of chloramphenicol treatment contained approximately 0.07 of the protein label in ribosomes and CM particles. After taking the second sample, the remaining third of the cells was filtered away from the chloramphenicol and resuspended in glucose minimal medium. After two hours of growth, their optical density was increasing exponentially and the third sample was taken. This contained approximately 0.03 of the protein label in ribosomes. Figure 8 shows the gradient profiles discussed and the uracil profiles which indicate the position of the CM particles and the mature ribosomes. There is clearly contaminating protein in the ribosome region, although it can be roughly estimated. After such an estimation, the corrected data are presented in Figure 9, which shows the protein activities of ribosomal protein before, during and after treatment with chloramphenicol. Figure 9 (lower) shows the specific activities, relative to total protein specific activity, of the proteins in question.

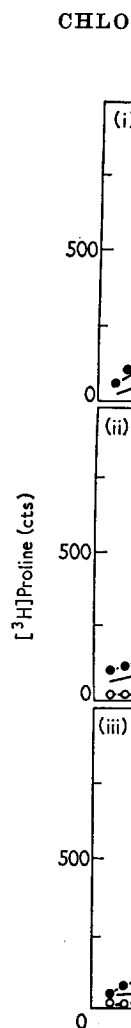


FIG. 8. (i) [ $^3\text{H}$ ]Proline (cts) in ribosomes and CM particles just before addition of chloramphenicol. The numbers shown represent the total counts in the region of the ribosomes and CM particles. The specific activity of ribosomes is estimated as 0.03. (ii) [ $^3\text{H}$ ]Proline (—●—●) in ribosomes and CM particles after 90 min of chloramphenicol treatment as shown in Fig. 6 (i). The numbers shown represent the total counts in the region of the ribosomes and CM particles. The specific activity of ribosomes is estimated as 0.065. (iii) After the 90-min CM treatment as shown in Fig. 6 (ii) and resuspended in glucose minimal medium. 2 h after harvest the profile shown in (ii) has entered ribosomes, and the specific activity of ribosomal protein can be estimated, since the ribosomes are now the major source of protein.



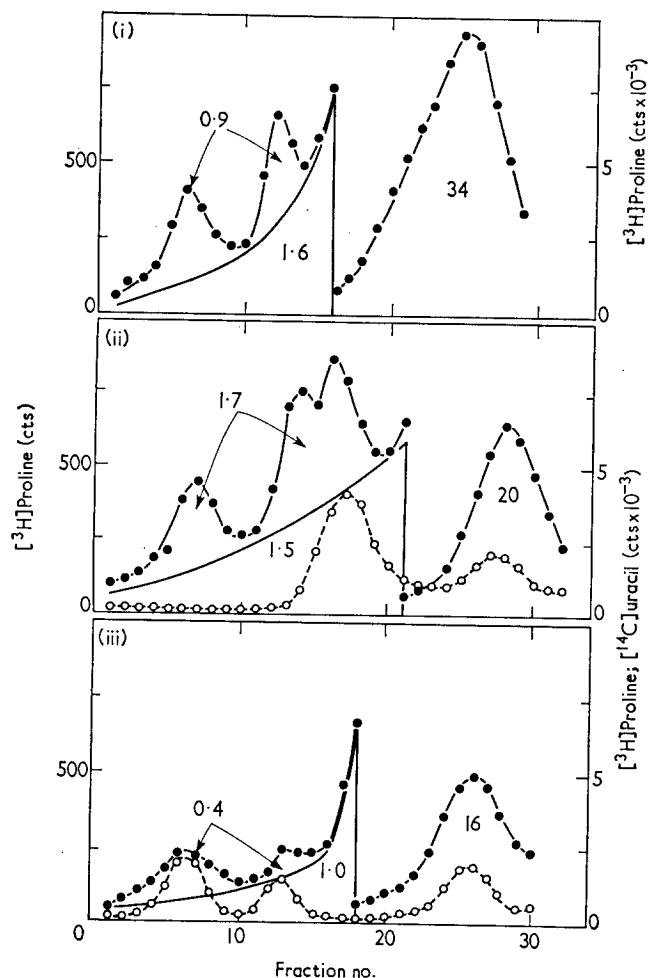


FIG. 8. (i)  $^3\text{H}$ Proline profile of cells after the treatment shown in Fig. 6 (i).

This sample was taken just before addition of CM. The amount of non-ribosomal protein contaminating the ribosomal region has been estimated in this and (ii) and (iii) by eye. The numbers shown represent the areas under the respective curves, and there is a scale change by a factor of 10 for proline counts in the middle of all three gradients. The fraction of radioactivity in ribosomes is estimated as  $0.09/365 = 0.025$ .

(ii)  $^3\text{H}$ Proline (—●—●—) and  $^{14}\text{C}$ uracil (—○—○—) profiles taken after 90 min of CM treatment as shown in Fig. 6 (i). The position of CM particles is shown by the uracil incorporated during the drug treatment. In the calculation of the fraction of radioactive material in ribosomes or chloramphenicol particles, the correction discussed in Materials and Methods for cell wall spun out with unbroken cells was applied. The fraction of radioactive material in ribosomes in this case was 0.065.

(iii) After the 90-min CM treatment, the remainder of the culture was filtered and resuspended in glucose minimal medium. 2 hr after resuspension the cells were growing normally and they were harvested for the profile shown here. The RNA labeled during the CM treatment (—○—○—) has entered ribosomes, and despite the contaminating non-ribosomal proteins, it can be seen that the activity of ribosomal protein (—●—●—) is approximately 0.024. The non-ribosomal protein can be estimated, since the ribosomal protein must parallel the ribosomal RNA profile.

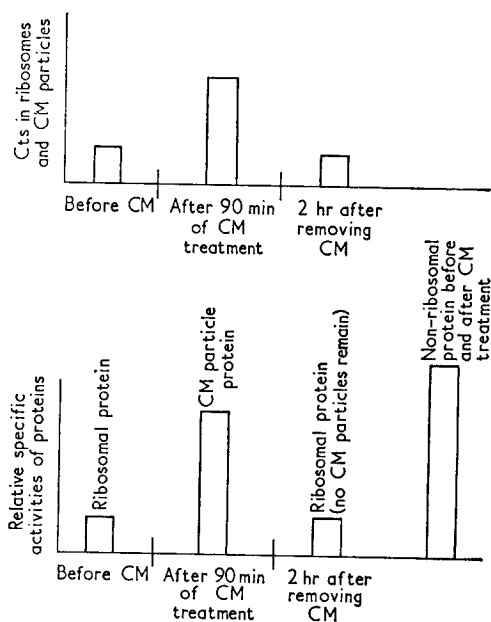


FIG. 9. Activity and specific activity relative to total protein specific activity of ribosomes and CM particles before treatment, after 90 min of CM treatment, and 2 hr after removing CM.

The specific activities relative to total cell protein were calculated as follows: for ribosomal protein before and after CM treatment, 0.025 of their activity divided by 0.15%, their fraction of cellular protein (Schleif, 1967a,b); for non-ribosomal protein, 0.97 of its activity divided by 0.85 fraction of its cell protein, and for CM particle protein, 0.04 of its activity divided by its estimated value, 0.06, of cellular protein in this case (roughly half the amount of ribosomal protein).

These experiments are interpreted as showing that the majority, more than 80%, of the proteins of CM particles is derived from pools of pre-existing non-ribosomal protein; and hence that CM particles are not a defined stage of ribosome maturation, with only a subclass of ribosomal proteins bound to ribosomal RNA. Instead they appear to be formed by association predominantly of non-ribosomal protein with the RNA synthesized during chloramphenicol treatment.

As the CM particles appear not to be a defined stage in ribosome maturation, but the result of association between their ribosomal RNA and non-ribosomal protein, it is expected that they would not be a homogeneous class. This expectation is fulfilled, for as shown by Hosokawa & Nomura (1965), the CM particles are heterogeneous and contain particles with a spread in their protein to RNA ratio.

The protein likely to associate with the RNA synthesized during chloramphenicol treatment would most probably be basic and resemble ribosomal protein in some physical properties. Therefore it is not surprising that gel electrophoresis of CM particle protein (Kaji & Nakada, 1967) or RC particle protein (subribosomal particles formed in a relaxed strain during amino acid starvation (Nakada, 1967)) shows the presence of basic proteins. The apparent identity, however, of several proteins from the subribosomal particles to proteins from mature ribosomes suggests that the CM or RC particles might contain several proteins also found on mature ribosomes. Nakada (1967) showed that the proteins of RC particles are synthesized before amino acid starvation, but concluded on the basis of similarity in electrophoretic patterns of ribosomal and RC proteins, that they were predominantly ribosomal proteins. In

addition to the expected proteins, further possible subparticle protein is shown in part being spread over the gel.

Previous studies on the ribosomes (Gavrilova & Spirin, 1967) or have not explored extensive ribosome precursors. The control ribosomes without replacement data presented here, as is the RC particles are similar to CM

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addition to the expected similarity of ribosomal and chloramphenicol particle proteins, further possible sources for this discrepancy are that only a subclass of RC particle protein is shown in protein bands of the electrophoretic separation, with the rest being spread over the gel or lost before electrophoresis.

Previous studies on the relationship between CM particles (Lerman, Zimmerman, Gavrilova & Spirin, 1967) or RC particles (Nakada & Unowsky, 1966) and ribosomes have not explored extensively the possibility that the particles in question are not ribosome precursors. The conclusion that CM particles could be directly matured to ribosomes without replacement of the CM particle protein is incompatible with the data presented here, as is the analogous conclusion for RC particles, if we assume that RC particles are similar to CM particles.

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