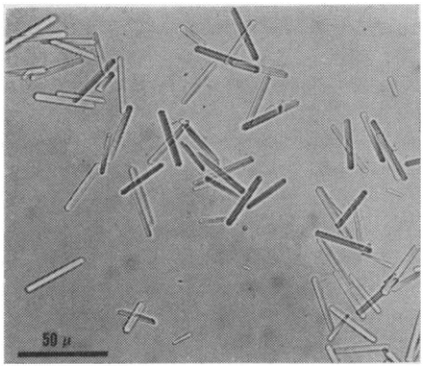

Fig. 2. Thrice recrystallized photoprotein (CPC) of *Chaetopterus*.

use of area-height and extrapolated zero time, as 3.04×10^{-7} for CPA and 2.80×10^{-7} for CPC.

From these data molecular weights of 128,000 for CPA and 184,000 for CPC were calculated. In all calculations the partial specific volume was assumed to be 0.73. Determination of molecular weight by Sephadex G-200 gave 110,000 for CPA. Both proteins were colorless and heat-labile, giving no evidence of peroxidase activity. One milliliter of solution having an optical density of 1.0 per centimeter at 280 mμ contained 0.48 mg of protein, dry weight.

In regard to specific requirements for bright luminescence, before separation of CP and cofactor 1 the only diffusible factors appeared to be Fe^{++}, O_2, and a hydroperoxide such as those that form spontaneously during storage of dioxane or tetrahydrofuran (*1*). We have found that tetrahydropyrane hydroperoxide, ethyl hydroperoxide, and dicumyl hydroperoxide have almost no activity in this system. With increasing purification of CP, luminescence activity progressively decreased in the presence of O_2 and optimum concentrations of cofactor 1, Fe^{++}, and dioxane. The activity could be restored, however, by addition of either a small amount of impure extract or (better) 0.1 to 0.2 mg/ml of an impure hyaluronidase preparation. With the purest photoprotein material the activation of luminescence in this way was more than 50-fold. The fact that more-highly purified hyaluronidase preparations gave less activation of luminescence indicated that the activating factor (cofactor 2) occurs as an impurity.

While its chemical nature remains unknown, cofactor 2 is heat-stable, undialyzable, resistant to acid and alkali, and soluble in 2:1 mixture of acetone and water. Perhaps it is a lipid. The activation mechanism appears to involve protection of photoprotein and cofactor 1 from nonluminescent destruction by Fe^{++} and peroxide, judged by progressive loss in activity during incubation in the absence of sufficient cofactor 2, and also by data on total light. The previously reported (*1*) catalytic activity of spent photoprotein solutions on the luminescence reaction of unspent solutions is most likely attributable to the presence in the former of cofactor 1 or 2, or both.

The kinetics of light-emission is subject to complex variation with varying proportions of components in the reaction mixture. The maximum total light is practically the same, however, for a given amount of CPA and CPC, with optimum amounts of the other components in each instance. Moreover the fact that under given conditions the maximum total light is proportional to the amount of photoprotein over a wide range of concentrations justifies the term photoprotein (*1*, *4*), although this relation does not hold with suboptimum concentrations of cofactor 2.

The maximum total light elicited from 0.043 mg of CPA or CPC at 25°C was 2×10^{12} photons. If one assumes the molecular weights of CPA and CPC to be 120,000 and 184,000, respectively, the quantum yields, or (number of photons emitted) : (number of photoprotein molecules reacted), amount to 0.0093 for the amorphous form and 0.0143 for the crystalline form. Since it is clear that the amorphous form (CPA) converts to the crystalline form in solution of ammonium sulfate, the ratio of molecular weights suggests that CPA is a dimer and CPC is a trimer of a monomeric form that is probably the most reactive form, inasmuch as we found by reaction kinetics that CPC is less reactive than CPA.

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12 February 1968

Ribosome Biogenesis: Nonrandom Addition of Structural Proteins to 50S Subunits

Abstract. *Assemblage of structural proteins into 50S subunits was examined in* Escherichia coli *recovering from chloramphenicol treatment. Cells previously labeled with H^3-leucine for three generations were incubated for 30 minutes with chloramphenicol. Proteins synthesized during the initial 5 minutes of recovery from chloramphenicol treatment were labeled with C^{14}-leucine. Marked variation in the ratios of C^{14}- to H^3-leucine in ribosomal protein occurred in cells that had been treated with chloramphenicol; untreated cells displayed little variation. The results suggest that ribosomal proteins are assembled into 50S subunits in a nonrandom manner.*

The studies of Waller (*1*), Leboy *et al.* (*2*), and Traub *et al.* (*3*) suggested that 70*S* ribosomes contain 30 structural proteins. Proteins extracted from 50*S* particles can be separated by polyacrylamide-gel electrophoresis into 20 different bands, while from the 30*S* particles 10 or 11 distinct bands can be distinguished. Little is known concerning the assemblage of these various proteins into ribosome particles. A number of cases exist in which bacterial cells previously incubated under conditions of inhibited protein synthesis preferentially synthesize ribosomal protein when protein formation is allowed to resume. *Escherichia coli* synthesized these structural proteins at a greater rate than soluble protein during recovery from chloramphenicol treatment

(*4*). Similar observations have been made with *E. coli* 15T recovering from puromycin treatment (*5*), *E. coli* K711 (RC^{rel}) recovering from methionine starvation (*6*), and *E. coli* B207 recovering from potassium depletion (*7*).

Our study was designed to determine whether each protein of the 50*S* particle is synthesized at the same rate during recovery from chloramphenicol treatment. Using a double-labeling technique, we compared synthesis of 50*S* ribosomal proteins (r-proteins) in exponentially growing cells with that in cells during recovery from chloramphenicol treatment. The results suggest that r-proteins appear on the 50*S* particle during the first 5 minutes of recovery from chloramphenicol treatment at rates different from those observed in exponentially growing cells. To eliminate problems of reproducible extraction of r-protein, all experiments were performed with cells that were labeled with H^3-leucine. During recovery from chloramphenicol treatment the synthesized r-proteins were labeled with C^{14}-leucine. When protein was extracted from the double-labeled cells, the various r-proteins contained both H^3 and C^{14}; thus, differences due to extraction procedures were eliminated.

The ribosomal protein synthesized in exponentially growing cells and that found in cells during the first 5 minutes of recovery after chloramphenicol treatment were compared in the following way. Cell crops of *Escherichia coli* K711 (met^-, arg^-) were incubated at 37°C in 1 liter of minimal medium (*8*) with glycerol (0.1 percent), L-methionine ($3.3 \times 10^{-4}M$), and L-arginine ($2.4 \times 10^{-4}M$). To uniformly label all protein, we incubated cells for three generations in the presence of L-leucine-4,5-H^3 (1.5 μc/ml, final concentration $1.1 \times 10^{-4}M$). When the suspension had reached an absorption of 0.8 at 575 nm, the cells (1 liter) were chilled by pouring them over crushed ice (500 g) held at −20°C. To a second liter of cell suspension, chloramphenicol was added to a final concentration of 100 μg/ml to inhibit protein synthesis. After these cells were incubated for 30 minutes in the presence of chloramphenicol, they were chilled in the manner described. Treated and untreated cells were collected by centrifugation at 4°C and suspended in 1 liter of warmed (37°C) media containing $4 \times 10^{-6}M$ L-leucine-1-C^{14} (0.04 μc/ml). Five minutes later, L-leucine was added to a final concentration of $4 \times 10^{-4}M$, and incubation was continued for 10 minutes to "chase" nascent soluble protein from the ribosomes. The cells were chilled and collected as indicated above, washed with cold TMA buffer (*3*) containing $10^{-2}M$ magnesium acetate [TMA ($10^{-2}M$ Mg^{++})], resuspended in 15 ml of this buffer, and frozen at −20°C.

The cells were broken at 0° to 3°C in a French pressure cell to isolate ribosomes. Sodium deoxycholate (1 mg/ml final concentration) was added, and 20 minutes later whole cells and cell debris were removed by centrifugation. To further purify the ribosomes, the supernatant material (15 ml) was layered over 12 ml of 10 percent sucrose in TMA ($10^{-2}M$ Mg^{++}) previously layered over 3 ml of 68 percent sucrose in this buffer. After centrifuging at 25,000 rev/min for 5 hours in an SW-25 rotor of a Spinco model L ultracentrifuge, the ribosomes in the lower one-third of the 10 percent sucrose and in the 68 percent sucrose were removed and dialyzed against two changes of 400 ml TMA ($10^{-4}M$ Mg^{++}) for 5 to 6 hours. The ribosomes were fractionated into subunits by two cycles of sucrose-gradient centrifugation as described by Traub *et al.* (*3*). The final 50*S* subunit preparation contained less than 6 percent contamination with 30*S* subunits. The 50*S* subunits were concentrated to at least 20 $O.D._{260\ nm}$ units per milliliter (*9*) by dialysis against solid sucrose.

The protein was extracted from the 50*S* ribosomal subunit with 67 percent acetic acid as described by Waller and Harris (*10*), dialyzed against 500 ml of distilled water, two 500 ml changes of 0.05*M* acetic acid with 0.001*M* EDTA (ethylenediaminetetraacetate), and finally dialyzed against 250 ml of 0.05*M* acetic acid containing 6*M* urea. The protein, determined by the method of Groves *et al.* (*11*), was concentrated with solid sucrose to approximately 2.5 mg/ml and separated by polyacrylamide-gel electrophoresis at *p*H 4.5 (25°C) in a system composed of 2.5 percent (weight/volume) acrylamide and 0.674 percent (w/v) ethylene diacrylate in the sample and spacer gels, and 10.0 percent (w/v) acrylamide and 0.50 percent (w/v) ethylene diacrylate in the resolving gel; all contained deionized 6*M* urea.

The protein was separated by polyacrylamide-gel electrophoresis (*12*) for 95 to 100 minutes at 2.5 ma per tube at room temperature, in glass tubes (80 by 5 mm) coated with silicone Dri-film. In each case, 70 μg of r-protein was applied. Gels were stained with 1 percent aniline blue black in a solution containing 7 percent acetic acid and 3 percent ethanol. A typical pattern of 50*S* r-protein is shown in Fig. 1.

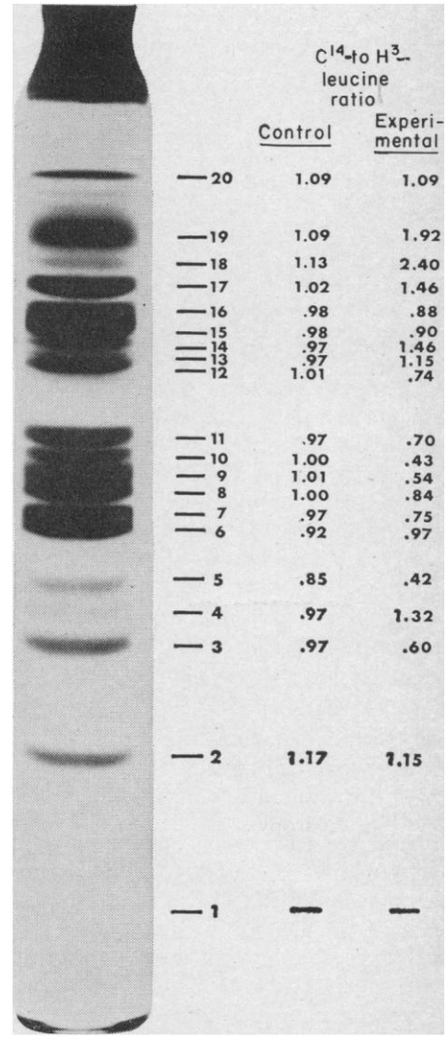

Fig. 1. Separation of labeled 50*S* ribosomal protein at *p*H 4.3 on a 10 percent polyacrylamide gel. The ratios of C^{14} to H^3 for the various ribosomal protein bands were calculated from Fig. 2, A and B. The mean of each of these groups was determined, and the results were normalized by dividing the appropriate mean into the ratios calculated from Fig. 2, A and B. As a control, exponentially growing cells were labeled for three generations with H^3-leucine for 5 minutes and then with C^{14}-leucine; in the experimental group, exponentially growing cells were labeled for three generations with H^3-leucine and for 5 minutes with C^{14}-leucine during recovery from a 30-minute treatment with chloramphenicol.

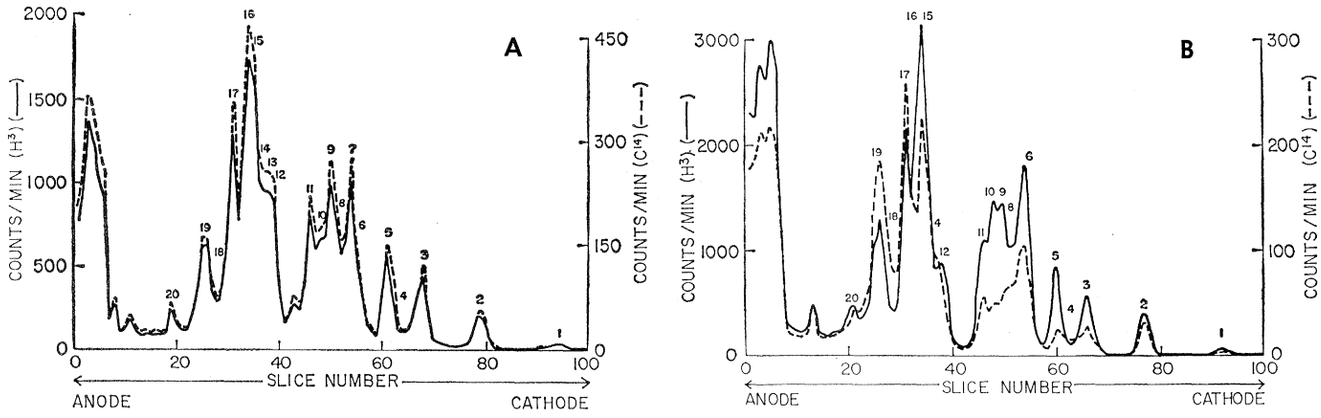

Fig. 2. Profiles of radioactivity of 50*S* ribosomal proteins (with and without chloramphenicol treatment) separated on 10 percent acrylamide gels. (A) *Escherichia coli* cells labeled for three generations with H^3-leucine followed by labeling for 5 minutes with C^{14}-leucine; and (B) *Escherichia coli* cells labeled with H^3-leucine for three generations and then labeled for 5 minutes with C^{14}-leucine during recovery from a 30-minute chloramphenicol treatment. The 50*S* ribosomal proteins were prepared and electrophoretically separated as indicated in the text. After electrophoresis the gels were cut into 1-mm slices (*15*), dissolved according to the method of Choules and Zimm (*16*), and counted in a Packard Tri-Carb liquid-scintillation counter. The numbers on the face of the figure indicate the individual protein bands.

The results of these experiments illustrated in Fig. 2, A and B, indicate that normal exponentially growing cells are labeled uniformly with C^{14}-leucine during a 5-minute incubation period, as shown by the coincidence of the C^{14}- and H^3-labeling patterns in Fig. 2A. During the first 5 minutes of recovery after chloramphenicol treatment, however, the labeling patterns obtained (Fig. 2B) of C^{14} and H^3 are not coincident and therefore suggest that there is variation in the labeling of r-protein during the recovery phase. An examination of these data reveals that there is little variation in the normalized ratios of C^{14} to H^3 ($\sim$ 1, Fig. 1) among the numbered protein bands in the untreated cells. Ratios obtained from cells labeled with H^3-leucine for three generations and with C^{14}-leucine during the first 5 minutes of recovery from 30 minutes of chloramphenicol treatment varied distinctly in the individual bands. These data indicate that the structural proteins of the 50*S* particle appear at different rates during the first 5 minutes of recovery. The same situation appears to obtain during the next 5 minutes of recovery, although the differences in the C^{14}/H^3 ratios of the various bands have diminished (unpublished observation).

A possible explanation for the differences in time of appearance of the various r-proteins on the 50*S* subunit is that during chloramphenicol treatment there are changes in the r-protein pools, resulting in degradation or accumulation of certain r-proteins to different extents during chloramphenicol treatment. Evidence, however, for the existence of a pool of r-proteins is indirect and not well documented. Some authors have suggested that, if such a pool exists, it is small (*13*). An analysis of the influence of chloramphenicol upon the integrity of ribosome particles was made by incubating labeled cells in a medium containing chloramphenicol. We were unable to detect selective release of particular structural proteins from the 50*S* ribosomes as a result of this treatment.

A second possibility is that, after addition of chloramphenicol, ribosome particles accumulate at various stages of maturation, in which certain structural proteins are lacking. After removal of the chloramphenicol, the missing proteins are synthesized during the recovery phase. If this interpretation is correct, the missing proteins would be labeled to different degrees during recovery, depending upon the sequence of addition in the assembly process. The final protein added would be labeled most heavily, since it must be added to all incomplete particles and should have the highest ratio of C^{14} to H^3. The proteins added prior to the last would be labeled to a lesser extent. This interpretation assumes that r-proteins are assembled into ribosomes in a nonrandom manner.

Recent work by Otaka, Itoh, and Osawa (*14*) indicated that *E. coli* cells incubated with a low concentration of chloramphenicol accumulate precursor particles. Among these is a 40*S* particle which contains all but four of the structural proteins of the 50*S* particle. With the use of higher concentrations of the drug, a series of precursor particles may accumulate, lacking different numbers of structural proteins, and during recovery the missing proteins may be synthesized preferentially to complete the precursor particles.

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17. Supported by grants from the Damon Runyon Memorial Fund (DRG–743), NSF (GB–5743), NIH (AM–07375), and by the American Lebanese Syrian Associated Charities (ALSAC). F.C.D. is a predoctoral trainee of NCI, grant, 5–T01–CA–5176.

6 November 1967; revised 15 December 1967