that the theorem was true for any compact Lie group. Our generalization requires the unpublished results of Atiyah for its proof.

The proof of the theorem is too long to describe here, though a few comments can be made on it. In his lecture notes from Harvard, Bott[3] describes an exact sequence involving the real and the complex K-theories of a space. This sequence, together with the results of Atiyah and Hirzebruch, will easily lead one to conjecture our theorem. Unfortunately, Bott's sequence will not quite suffice to prove the theorem for anything more complicated than a torus, and then only by very *ad hoc* methods. Our proof proceeds by establishing a new form of K-theory, and proving several relationships between this K-theory and the two older theories. These relationships take the form of exact sequences similar to Bott's sequence. By proving that similar sequences are exact for the completed representation rings, one obtains the theorem from repeated applications of the five lemma.

The new K-theory, which we denote by KC^*, takes as its fundamental object pairs (E, e), where E is a complex vector bundle which is self-conjugate, and e is a bundle equivalence between E and its conjugate. KC^* is a periodic theory, and the periodicity arises by taking cup products with the generator of KC^{-4} (point). The coefficient ring KC^* (point) is generated by four elements x_i of dimension i, for $i = 1, 3, 4$, and -4. The relations among these generators are:

$$2x_3 = 0, \qquad (x_1)^2 = (x_3)^2 = x_1x_3 = 0, \qquad x_4x_{-4} = 1.$$

[1] Atiyah, M. F., "Characters and cohomology of finite groups," Publ. Math. No. 9 (Institut des Hautes Études Scientifiques, 1961).

[2] Atiyah, M. F., and F. Hirzebruch, "Vector bundles and homogeneous spaces," *Differential Geometry*, Proceedings of Symposium in Pure Mathematics, Amer. Math. Soc., vol. 3 (1961).

[3] Bott, R., Lectures on $K(X)$, mimeographed notes, Harvard 1962.

[4] Milnor, J., "Construction of universal bundles I," *Ann. of Math.*, **63**, 272–284 (1956).

---

*FUNCTIONAL RIBOSOMES IN ANTIBODY-PRODUCING CELLS**

BY KURT H. STENZEL,† WILLIAM D. PHILLIPS, DAVID D. THOMPSON, AND ALBERT L. RUBIN

ROGOSIN LABORATORIES, DEPARTMENT OF MEDICINE, THE NEW YORK HOSPITAL-CORNELL MEDICAL CENTER, AND DEPARTMENT OF BIOLOGY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, AND CENTRAL RESEARCH DEPARTMENT, E. I. DU PONT DE NEMOURS AND COMPANY

Communicated by Francis O. Schmitt, February 19, 1964

Ribosomal aggregates (polysomes) have recently been found to be functional units for the incorporation of amino acids into peptide chains during protein synthesis in some mammalian,[1–3] bacterial,[4] and plant[5] cells. Present evidence indicates that the aggregates are held together by messenger RNA.[1,6] The messenger RNA chain length is thought to determine the size both of coded polypeptide chains and of active ribosomal aggregates.[1] Antibody-producing cells are unique in that, although they produce the same general class of proteins (immunoglobulins), the combining characteristics of the particular globulins produced are markedly altered by specific antigenic stimuli. A knowledge of the functional ribosomal unit involved

in the production of immunoglobulins may provide insight into the mechanisms of antibody production.

The present experiments were designed to determine the ribosomal units associated with active synthesis of polypeptides in antibody-producing cells, and to determine whether possible differences in these units are associated with synthesis of different antibodies.

Materials and Methods.—New Zealand white female rabbits were hyperimmunized with either horse spleen ferritin or human plasma albumin.[7] Increasing amounts of alum-precipitated antigen (15 mg antigen N/100 ml) were administered intravenously 3 days per week for 4 weeks until each animal had received a total of 3 mg antigen N. Antibody titers were determined by passive hemagglutination with tanned, sensitized sheep erythrocytes.[8]

Spleens were removed from immunized and nonimmunized rabbits under ether anesthesia, and immediately placed in iced buffer A (0.14 M NaCl, 5×10^{-3} M $MgCl_2$, 10^{-2} M Tris, pH 7.4). They were then minced, passed through a fine stainless steel screen, washed twice by centrifugation at 1,000 rpm in buffer A, and cell counts performed. Cells were again centrifuged and resuspended in buffer B (10^{-2} M KCl, 1.5×10^{-3} M $MgCl_2$, 10^{-2} M Tris, pH 7.4), at a concentration of 10^8 nucleated cells/ml, and gently disrupted in a glass Dounce tissue homogenizer. The resulting homogenate was centrifuged at 1,500 rpm for 10 min to remove nuclei and cell debris. The supernate was then carefully removed, and 0.1 volume 5% deoxycholate added to it. This material is designated the crude ribosomal fraction. One-ml aliquots were layered on linear 15–30% (w/w) sucrose gradients in buffer B and centrifuged at 24,000 rpm (SW 25 rotor of Spinco Model L ultracentrifuge) for 90–120 min. The bottoms of the tubes were then punctured, 30–40 fractions collected from each tube by drop counting, and the optical density at 260 mμ was determined. Temperature was carefully maintained at 0–1°C throughout.

To inhibit RNase activity, Roussin salts[9] ($[Fe_4S_3(NO)_7]K$) 8×10^{-5} M were added to buffer B and sucrose solutions in one experiment, and all solutions were pretreated with Bentonite in another.

In vitro incorporation of C^{14}-labeled amino acids was accomplished by incubating spleen cells in buffer A with 20% normal rabbit serum for 15 min at 37°C and then pulse-labeling for 3–5 min with 20 μc/ml C^{14} algal hydrolysate (1.38 mc/mg). The reaction was stopped by the addition of one volume iced buffer A. Cells were then washed twice in buffer A, and the crude ribosomal fraction was prepared as above. *In vivo* incorporation of C^{14}-phenylalanine (297 mc/mm) was accomplished by injecting 50 μc intravenously 5 min prior to splenectomy, and of C^{14}-uridine (30.0 mc/mm) by injecting 100 μc intravenously 30 min prior to splenectomy. After collection of fractions from the sucrose gradients and determination of the optical density at 260 mμ, 0.2 mg/ml bovine serum albumin was added to each tube, and fractions were precipitated with 5% trichloroacetic acid (TCA). Precipitates were then layered on 0.45-μ Millipore filters, and radioactivity was determined in a Nuclear-Chicago gas flow counter. All radioactive materials were obtained from New England Nuclear Corp.

For some studies, 0.1 volume of a 10 γ/ml solution of RNase (Sigma Chemical Co.) was added to the crude ribosomal fraction prior to density gradient centrifugation.

Polysome stability in the presence of rabbit spleen crude ribosomal fractions was assessed by two experiments. In the first, the crude ribosomal fraction from the spleen of an immunized rabbit was mixed with rabbit reticulocyte[10] ribosomes which had been prepared in the usual manner for preservation of polysomes.[1] In the second, the rabbit spleen ribosomal material was mixed with a similar preparation, prepared according to the method of Penman *et al.*[12] from HeLa cells that had been incubated overnight with C^{14}-uridine. These mixtures were then layered on sucrose gradients and centrifuged as described above.

To demonstrate production of antibody by the cells, 10^6 nucleated spleen cells/ml were incubated in medium 199 (Grand Island Biologicals) with 1% fresh glutamine and 30% fetal calf serum for 6 hr. Cells were removed by centrifugation at 1,000 rpm for 10 min, and 0.1-ml aliquots of the supernate precipitated by 5% TCA. Similar aliquots were added to reaction mixtures containing specific antigen and antibody (both unlabeled) at equivalence, and the radioactivity in the specific precipitate was measured after repeated washing. An approximate sedimentation velocity for the antibody was determined by sucrose density centrifugation.[11]

Results.—At the time of sacrifice, all immunized animals had antibody titers of 1:1000 or more as determined by passive hemagglutination. Density gradient centrifugation of the crude ribosomal fraction from spleen cells of rabbits hyperimmunized with either horse spleen ferritin or human plasma albumin revealed a characteristic pattern showing one broad, and in some instances biphasic, peak of optical density close to the meniscus (Fig. 1). Although a precise sedimentation

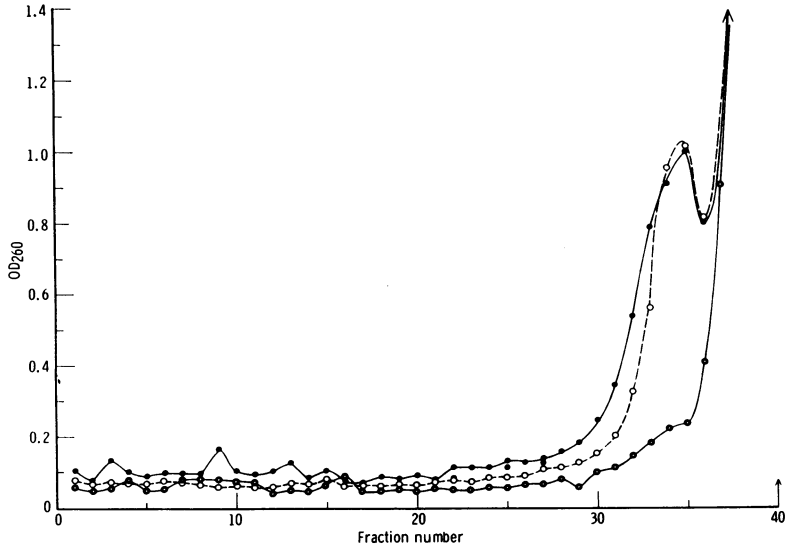


FIG. 1.—Sucrose gradients (15–30%) of crude ribosomal fractions from spleen cells of ferritin (●), albumin (○), and nonimmunized (○) rabbits centrifuged at 24,000 rpm in the SW 25 rotor of a Spinco Model L ultracentrifuge for 90 min. The arrow indicates the last fraction collected.

coefficient cannot be assigned to this peak, its position in the sucrose gradients when compared with sedimentation patterns of *E. coli* ribosomes at 10^{-2} and 10^{-3} *M* Mg^{++} indicates that it is in the 80–120S region, and is probably composed of ribosomal monomers and dimers. Results were identical whether the experiments were done in the presence of Roussin salts (8×10^{-5} *M*), or whether all solutions were pretreated with Bentonite. Furthermore, the crude ribosomal fractions of immunized rabbits' spleen cells did not cause degradation of rabbit reticulocyte or HeLa cell polysomes (Fig. 2). Ribosomal fractions from comparable numbers of nonimmunized rabbit spleen cells showed only a suggestion of an optical density peak in this area (Fig. 1). RNase-treated ribosomes from immunized animals again sedimented as a single peak which, however, was taller and sharper than before (Fig. 3*b*).

In vitro and *in vivo* pulse-labeling of spleen cells with amino acids resulted in a broad, biphasic peak of TCA-precipitable radioactivity in the region of maximum optical density. After treatment with RNase, only a single peak was seen (Fig. 3*a* and *b*).

A 30-min *in vivo* pulse with C^{14}-uridine revealed a small but significant incorporation of the isotope in the region of peak optical density, again in the 80–120S portion of the gradient.

When spleen cells from immunized rabbits were incubated in a suitable culture media with C^{14} amino acids, TCA-precipitable radioactive material was synthesized. After 6 hr of incubation, the amount of radioactive material precipitated with authentic antigen and antibody accounted for 90 per cent of the TCA-precipitable radioactive material in the culture media. Sedimentation velocity of both serum antibody and of that produced *in vitro* was 7S as estimated by sucrose density gradient centrifugation.

Discussion.—The evidence presented clearly demonstrates that there is an increase in ribosomes sedimenting at 80–120S from spleen cells of hyperimmunized

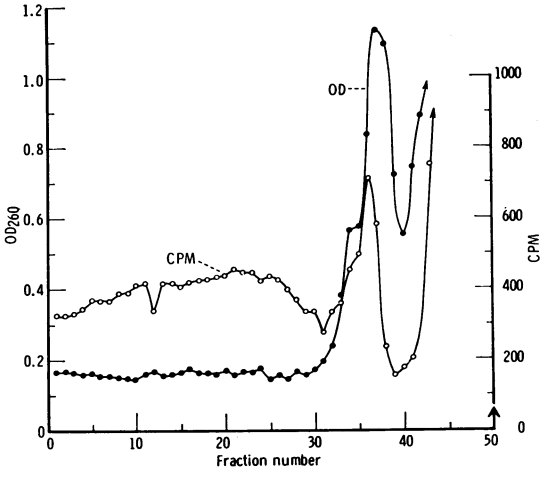
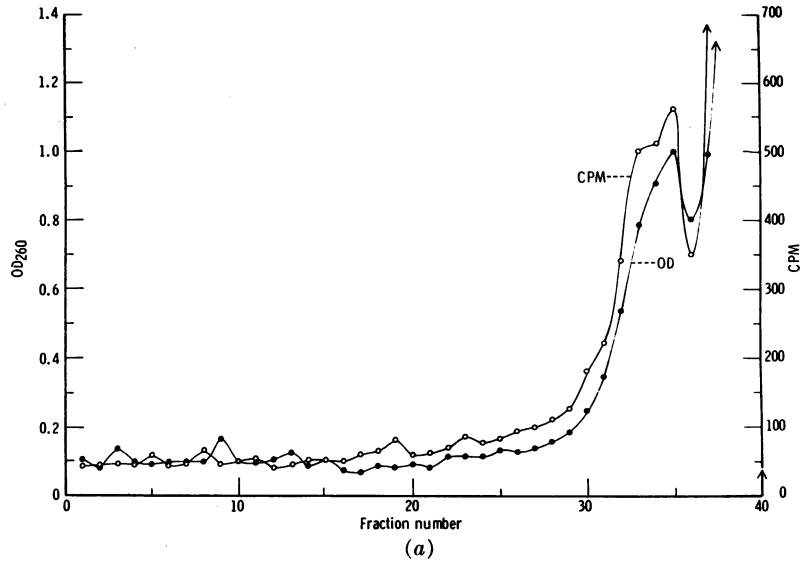


FIG. 2.—HeLa cells, incubated overnight in the presence of C^{14}-uridine, were broken in a Dounce tissue homogenizer, and the ribosomes mixed with the crude ribosomal fraction from spleen cells of a rabbit that had been hyperimmunized with albumin. The mixture was layered on a 15–30% sucrose gradient and centrifuged for 120 min at 24,000 rpm. After determination of OD_{260}, fractions were precipitated with 5% TCA in the presence of 0.8 mg carrier RNA, and radioactivity of the precipitates was determined (see text). The major contribution to OD is from the spleen cells. The labeled HeLa cell ribosomes clearly show a broad polysomal distribution.

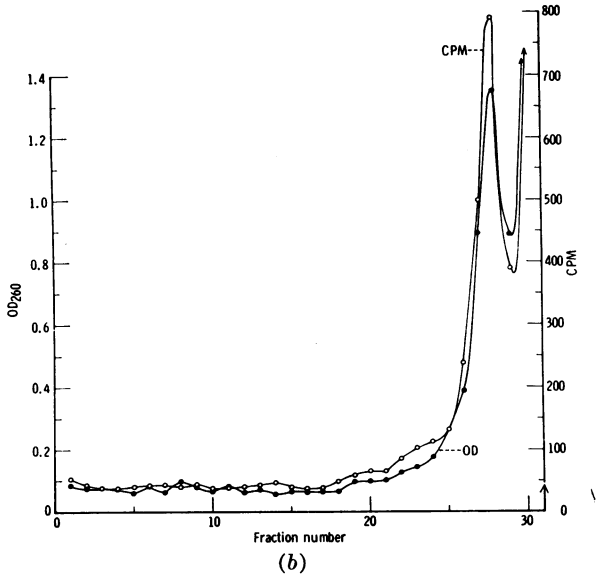
rabbits. The sedimentation patterns in sucrose density gradients of ribosomes from spleen cells of animals immunized with two different antigens are similar. This similarity might be common to cells producing a wide variety of antibodies.

Other types of protein-synthesizing mechanisms thus far investigated in animal and bacterial cells[1–4] indicate that ribosomal aggregates are the active sites for these processes. Single ribosomes appear notably inactive in this respect. In our studies, however, incorporation of amino acids into peptide chains appear to be associated with ribosomal monomers and dimers. The radioactivity in the monomeric peak could conceivably be associated with ribosomal, rather than nascent protein, although this would be unusual in view of the short pulse-labeling.

Splenic RNase activity could break up any possible polysomes in the rabbit spleen cells leaving only single and double ribosomes with nascent peptide chains remaining attached to them. Several experiments, however, indicate that the ribosomal dimers, and perhaps monomers, are indeed the active units. The presence of high con-



(a)



(b)

FIG. 3.—Sucrose gradients of crude ribosomal fractions from spleen cells of an albumin immunized rabbit pulsed *in vitro* for 5 min with 20 μc/ml C^{14} algal hydrolysate; (*a*) no RNase added, and (*b*) with 1 γ/ml RNase.

centrations of Roussin salts and pretreatment of all reagents with Bentonite did not alter the sedimentation patterns; labeled RNA appeared in the monomer and dimer region after a short (30-min) pulse with C^{14}-uridine and 1 γ/ml RNase at 0°C caused a consistent decrease in the 120S and an increase in the 80S peak. Finally, the clearest demonstration that the appearance of monomers and dimers is not the result of RNase activity is the failure of splenic ribosomal extracts to disrupt the sedimentation patterns of either rabbit reticulocyte or HeLa cell polysomes. It is

unlikely that the moderate trauma of shearing in a Dounce homogenizer could completely disrupt all ribosomal aggregates, since similar procedures with other mammalian cells have clearly preserved polysomes.[12]

There is no direct evidence that the peptide chains synthesized on ribosomes in our studies are antibody or antibody subunits. They could conceivably be subunits of other proteins involved in cellular metabolic activities. However, rapid appearance of labeled antibody in the culture media when these cells are incubated *in vitro* and the observation that specific antibody accounts for 90% of the protein produced, support the concept that the observed amino acid incorporation on ribosomes is in antibody. Work is now in progress to identify specifically this nascent protein material.

Our experiments show that a rapidly labeled and extremely RNase-sensitive RNA is associated with the active ribosomes. The assumption that this is messenger or template RNA may be correct, and further elucidation of its nature should allow valuable insight into the mechanisms and specificity of antibody synthesis.

If the functional ribosomal units in antibody-producing cells are monomers and dimers associated with an RNA possessed of template activity, the coded polypeptide chain may be of low molecular weight. Warner *et al.*[1] pointed out that in ribosomal aggregates from reticulocytes, there is 50–100 Å between the 230Å diameter ribosomes. Thus, a template RNA binding one or two ribosomes would be at most 560 Å long. Assuming a trinucleotide code with 3.4 Å per nucleotide, a messenger of 560 Å would code for only about 50–60 amino acids. It is difficult to reconcile this with current molecular models of gamma globulin[13] in which the subunit chain lengths are thought to have molecular weights of 20,000 and 60,000. Either these geometric considerations are not applicable to the special case of gamma globulin synthesis and a much longer messenger is associated with the active ribosomes, or the basic polypeptide unit of gamma globulin is smaller than heretofore recognized.

The latter possibility suggests a novel concept for the genesis of antibody specificity. The synthesis of small polypeptide chains may allow for diverse secondary and tertiary structure of antibody molecules by a variable combination of these subunits. Thus, while not excluding the possibility of differences in primary structure among antibodies, this concept would emphasize the combination of subunits, with consequent alterations in the configuration of the final molecule, as the method for development of specificity. The specific combination may take the form of a selection from a restricted population of such subunits.

Summary.—Evidence is presented which suggests that antibody is synthesized in association with ribosomal monomers and dimers, as well as with a rapidly labeled RNA. Similar sucrose density gradient sedimentation patterns are observed with ribosomes from spleens of rabbits immunized with two different antigens. A mechanism for antibody specificity based on a variable combination of small subunits is suggested.

The authors wish to thank Professor F. O. Schmitt and Dr. Peter F. Davison of the Department of Biology, Massachusetts Institute of Technology, for many helpful discussions.

* This work was supported by grants from the U.S. Public Health Service (HE08736), Health Research Council of the City of New York (U1022), American Heart Association, and New York Heart Association.

† Fellow of the New York Heart Association.

[1] Warner, J. R., P. M. Knopf, and A. Rich, these PROCEEDINGS, **49**, 122 (1963).
[2] Gierer, A., *J. Mol. Biol.*, **6**, 148 (1963).
[3] Wettstein, F. O., T. Staehlin, and H. Noll, *Nature*, **197**, 430 (1963).
[4] Gilbert, W., *J. Mol. Biol.*, **6**, 374 (1963).
[5] Clark, M. F., R. E. F. Matthews, and R. K. Ralph, *Biochem. Biophys. Res. Comm.*, **13**, 505 (1963).
[6] Warner, J. R., A. Rich, and C. E. Hall, *Science*, **138**, 1399 (1962).
[7] Horse spleen ferritin was kindly supplied by Dr. A. Mazur, and human plasma albumin was prepared in our laboratory by curtain electrophoresis.
[8] Daniel, T. M., J. G. M. Weyland, Jr., and A. B. Stavitsky, *J. Immunol.*, **90**, 741 (1963).
[9] Dobry-Duclaux, A., *Biochim. Biophys. Acta*, **38**, 33 (1960).
[10] Rabbit reticulocytes were kindly supplied by Dr. John R. Warner.
[11] Kunkel, H. G., in *The Plasma Proteins*, ed. F. W. Putman (Academic Press, 1960), vol. 1, p. 279.
[12] Penman, S., K. Scherrer, Y. Becker, and J. E. Darnell, these PROCEEDINGS, **49**, 654 (1963).
[13] Edelman, G. M., D. E. Olins, J. A. Gally, and N. D. Zinder, these PROCEEDINGS, **50**, 753 (1963).

EVIDENCE FOR DEGENERACY AND AMBIGUITY IN INTERSPECIES AMINOACYL-sRNA FORMATION

BY W. EDGAR BARNETT AND K. BRUCE JACOBSON

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY,* OAK RIDGE, TENNESSEE

Communicated by Alexander Hollaender, February 18, 1964

The role of transfer or sRNA in protein synthesis has been elucidated and amply reviewed.[1-3] Distinct sRNA species for the different amino acids have been separated,[3, 4] and amino acid specific aminoacyl-sRNA synthetases have been partially purified.[5] As adaptor molecules[6] the various sRNA's are direct intermediates involved in positioning amino acids in a correct linear sequence for polymerization into protein molecules. A prerequisite in assuring the correct positioning of an amino acid, therefore, is that the activated amino acid-synthetase complex recognize the correct sRNA molecule for aminoacyl-sRNA formation. Thus, each species of sRNA must have a site or recognition unit which distinguishes it from all other species of sRNA. In this report, recognition unit will refer to that series of nucleotides which is responsible for amino acid specificity, and references to coding will refer exclusively to these recognition units.

Data are presented in this report which indicate that the code used in aminoacyl-sRNA recognition units is not universal and which demonstrate interspecies degeneracy and ambiguity.

Materials and Methods.—(a) *Strains and media: Neurospora crassa* wild-type strain 74A-OR (provided by Dr. F. J. de Serres) and *E. coli* B were used. Neurospora cultures were grown on Westergaard's and Mitchell's[7] minimal medium containing 1% glucose. *E. coli* was grown as described by Nishimura and Novelli.[8]

(b) *Preparation of enzyme fractions:* Hyphae of Neurospora in the exponential phase of growth were collected on cheesecloth, washed once in cold distilled water, and immediately frozen in liquid nitrogen. The cells were disrupted by grinding in an electric mortar and pestle while frozen in liquid nitrogen. The resulting frozen powder was suspended in four volumes of medium A containing: 0.05 *M* Tris-HCl buffer, pH 7.7; 0.004 *M* magnesium acetate; 0.0125 *M* KCl; 0.01 *M*