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*BIOSYNTHESIS OF THE COAT PROTEIN OF COLIPHAGE f2 BY EXTRACTS OF EUGLENA GRACILIS**

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Viral RNA stimulates the incorporation of C^{14}-amino acids into protein in cell-free extracts of *E. coli*.[1-4] Although the nucleic acids of f2 bacteriophage and tobacco mosaic virus (TMV) direct the synthesis of characteristic products,[1, 2] identifiable virus coat protein is formed only in response to the RNA isolated from the bacteriophage.[1, 5]

Lack of success in obtaining synthesis of recognizable plant virus protein in bacterial extracts raises the question of whether some incompatibility might exist between the components of protein synthesis in distant species. Some species specificity has been demonstrated for activating enzymes and sRNA's[6] and for the aminoacyl-sRNA transfer factor;[7] nevertheless, von Ehrenstein and Lipmann[8] have shown that aminoacyl-sRNA from *E. coli* serves adequately in the synthesis of hemoglobin in extracts from reticulocytes of the rabbit.

The observation that amino acid incorporation into protein by chloroplast ribosomes of *Euglena gracilis* is markedly stimulated by exogenous RNA[9] permits us to test whether the information contained in the RNA of one species can be translated correctly during protein synthesis in extracts of another species. We report here experiments which indicate that RNA isolated from the f2 bacteriophage, known to direct the synthesis of its coat protein in extracts of *E. coli*, also leads to the synthesis of f2 coat protein in extracts of *Euglena gracilis*.

Materials and Methods.—Viral RNA and proteins: f2 Bacteriophage RNA and protein were prepared by shaking suspensions of purified virus with phenol.[1] TMV RNA and protein were prepared in the same way from virus purified by differential centrifugation of extracts from the leaves of young, infected tobacco plants.[10] The infected plants were the gift of Dr. F. O. Holmes of the Rockefeller Institute. RNA concentration was determined by measuring the optical density at 260 mμ and assuming a specific absorption of 24 per mg.

Cell-free extracts: Chloroplasts were purified from *Euglena gracilis*, strain Z, grown with carbon dioxide as the sole carbon source.[9] Ribosomes were obtained from lysed chloroplasts by differential centrifugation at 23,000 × *g* and at 100,000 × *g*. The crude ribosome pellet was resuspended in a small volume of 0.01 *M* Tris-HCl buffer at pH 7.6 containing 2×10^{-3} *M* $MgCl_2$, and the suspension was centrifuged at 3,000 × *g* for 10 min. Both the resulting supernatant and sediment contained active ribosomes, but the sediment contained a greater amount of contaminating protein.[9] Both of these ribosome fractions were used in the incorporation experiments.

Preparation of the supernatant fraction from *E. gracilis* and the conditions for the incorporation of amino acids into protein with cell-free extracts of *E. gracilis* have been described elsewhere in detail.[11] The amount of supernatant fraction used in these experiments was approximately 1 mg of protein per ml of incubation.

Cell-free (S-30) extracts from *Escherichia coli* B were prepared and preincubated according to the methods of Nirenberg and Matthaei.[12]

Measurement of RNA and protein concentration in cell-free extracts was performed by methods described previously.[13]

For the incorporation experiments which are described in the legends of the figures, incubations were performed at 37° with C^{14}-leucine (31 mC/mmole) or with C^{14}-lysine and C^{14}-arginine (100–200 mC/mmole). Uniformly labeled amino acids were obtained from New England Nuclear Corp., Boston, Mass. The amount of amino acid incorporated into protein was estimated by taking small samples onto filter paper disks which were then heated with trichloroacetic acid (TCA) and prepared for scintillation counting by a modification of the method of Mans and Novelli.[14]

Identification of the radioactive product: The radioactive product was precipitated from the incubation mixtures by the addition of an equal volume of 10% TCA. The precipitate was kept at 90° in 5% TCA for 15 min, washed twice with 5% TCA, and then dried with two washes of ethanol-ether, and two washes of ether alone. Authentic coat proteins from both f2 bacteriophage and TMV were treated with TCA and the organic solvents in the same way.

The dried material, containing the radioactive products, was oxidized with performic acid (0.06 ml of performic acid for each mg of dried protein) by a modification of the method of Hirs, Moore, and Stein.[15] In some experiments an amount of virus coat protein equal to 10 times the protein content of the radioactive product was added, and the mixture was oxidized. About 15 mg of oxidized protein were hydrolyzed for 3 hr at room temperature in 1 ml of 0.05 *M* ammonium bicarbonate pH 7.9 containing 4% trypsin by weight of substrate. Salt-free, two times recrystallized trypsin was obtained from Worthington Biochemical Co., Freehold, N. J. After lyophilization to dryness, the hydrolysate was dissolved in the pH 4.7 electrophoresis buffer, and 20-μl samples were applied to paper. Peptides were separated by two-dimensional electrophoresis, as described previously.[1] Radioautography was performed with clinical X-ray film (Royal Blue, Eastman Kodak, Rochester, N. Y.). Electropherograms were stained by dipping in 0.5% ninhydrin in acetone; the ninhydrin color was stabilized by a second dip in acidified copper nitrate in acetone.[16]

Results.—The effect of increasing concentration of viral RNA on the amount of leucine incorporated into protein by extracts of *Euglena gracilis* and of *Escherichia coli* is shown in Figure 1. Incorporation of the radioactive amino acid is directly proportional to the RNA concentration up to 1.5 mg of RNA per ml. The *E. coli* extract was found to be 2.5 times more sensitive to stimulation by f2 RNA than was the extract from Euglena. TMV RNA stimulated chloroplast ribosomes about as effectively as did the RNA of f2.

Following performic acid oxidation and trypsin hydrolysis, the products of the stimulated extracts were compared by means of two-dimensional electrophoresis, a method previously used to study the biosynthesis of the f2 coat protein in extracts of *E. coli*.[1] A radioautogram of the tryptic peptides from the radioactive product of an *E. coli* extract stimulated by f2 RNA is shown in Figure 2. The pattern is quite similar to that of the coat protein published previously.[1, 2] The radioautogram shown in Figure 3 of the tryptic peptides from extracts of Euglena stimulated

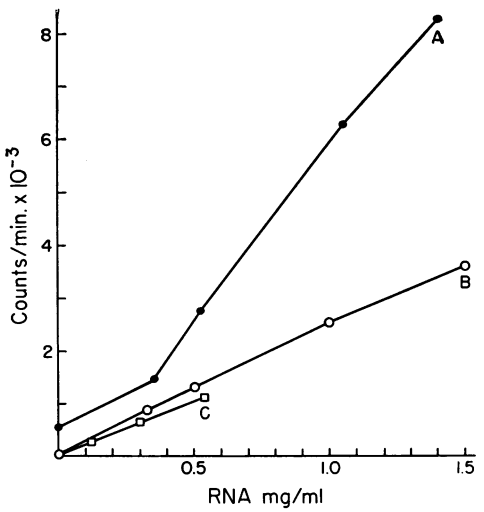

FIG. 1.—Effect of viral RNA concentration on the incorporation of C^{14}-leucine by extracts of *Euglena gracilis* and *Escherichia coli*. (*A*) *E. coli* extract with f2 RNA. The incubation contained 0.4 mg of ribosomal RNA per ml. (*B*) Euglena extract with f2 RNA. The incubation contained 0.23 mg of chloroplast ribosomal RNA per ml. (*C*) Euglena extract with TMV RNA. The incubation contained 0.23 mg of chloroplast ribosomal RNA per ml. All incubations were at 37° for 45 min.

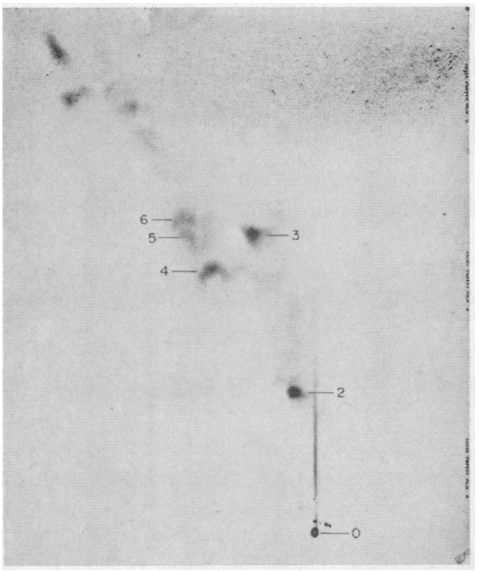

FIG. 2.—Radioautogram of an electropherogram of the tryptic peptides from the C^{14}-lysine- and C^{14}-arginine-labeled product stimulated by f2 RNA in an *E. coli* extract. The 3-ml incubation mixture contained per ml: 1.2 mg of ribosomal RNA; 0.93 mg of f2 RNA; 2.5 μC each of C^{14}-lysine and C^{14}-arginine, both with a specific radioactivity of 100 mC/mmole. In 60 min of incubation at 37°, a total of 344,000 cpm were incorporated into protein. *O* indicates the origin. Peptides are numbered as previously in Nathans *et al.*[1]

by f2 RNA reveals essentially the same pattern. Peptides numbered 2–6 in the radioautogram of the product synthesized in the *E. coli* extract (Fig. 2) are clearly evident in the radioautogram of the product from the Euglena extract (Fig. 3). The carboxyl terminal peptide of the coat protein, peptide 1, cannot be detected in either radioautograph. This peptide, which appears in electropherograms on staining with ninhydrin, does not contain lysine or arginine,[1] as expected from the specificity of trypsin.

Peptides 7 and 8 are not well resolved in either electropherogram. Some smearing of peptides is seen in both figures, and this is due to the large amount of protein that contaminated the products. Radioactive product from two additional extracts of Euglena stimulated by f2 RNA was also examined with f2 coat protein protein added as internal standard. The chloroplast ribosomes in these experiments were obtained from the supernatant of the final low-speed centrifugation described in *Materials and Methods*. Peptides from the radioactive product corresponding to numbers 2–9 were found to be congruent with the peptides of the carrier coat protein, which were stained with ninhydrin. Since these electropherograms were difficult to reproduce photographically, they are not shown.

When the RNA from TMV was used to stimulate Euglena extracts, an unidentifiable product was found (Fig. 4). No correspondence was seen between the pattern of radioactive peptides from this product and the peptides from TMV coat protein

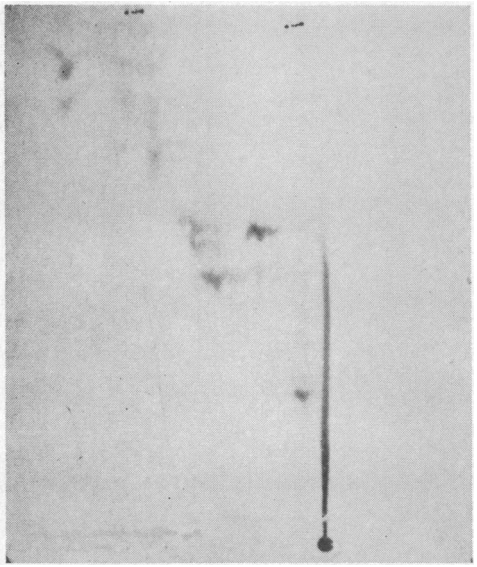

FIG. 3.—Radioautogram of an electropherogram of the tryptic peptides from the C^{14}-lysine- and C^{14}-arginine-labeled product stimulated by f2 RNA in an extract of *Euglena gracilis*. The 5-ml incubation mixture contained per ml: 0.7 mg of chloroplast ribosomal RNA; 1.12 mg of f2 RNA; 2.5 μC each of C^{14}-lysine and C^{14}-arginine, both with a specific radioactivity of 100 mC/mmole. In 60 min a total of 160,000 cpm were incorporated into protein. Chloroplast ribosomes used in this experiment were obtained as a pellet from the final low-speed centrifugation described in *Materials and Methods*.

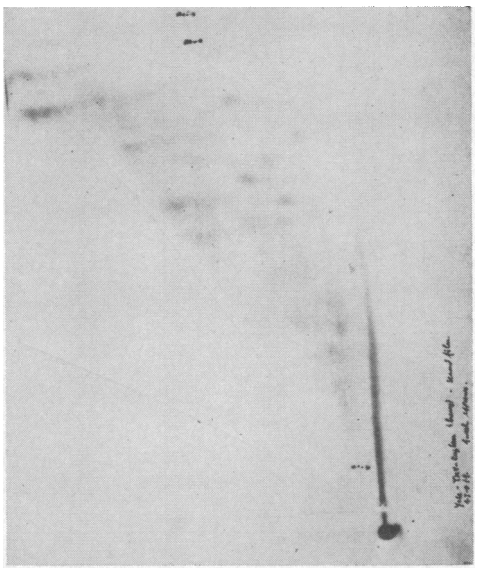

FIG. 4.—Radioautogram of an electropherogram of the tryptic peptides from the C^{14}-lysine- and C^{14}-arginine-labeled product stimulated by TMV RNA in an extract of *Euglena gracilis*. The 2-ml incubation mixture contained per ml: 0.71 mg of chloroplast ribosomal RNA; 0.92 mg of TMV RNA; 2.5 μC each of C^{14}-lysine and C^{14}-arginine, both with a specific radioactivity of 100 mC/mmole. In 20 min a total of 78,500 cpm were incorporated into protein. Chloroplast ribosomes used in this experiment were from the same preparation as those referred to in the legend of Fig. 3.

added as internal standard. Both *E. coli* extracts[1, 2, 5] and Euglena extracts fail to synthesize detectable amounts of TMV coat protein.

Discussion and Conclusions.—Analyses of the radioactive product synthesized in extracts of *Euglena gracilis* in the presence of bacteriophage RNA indicate that the primary structure of the f2 coat protein is correctly assembled upon chloroplast ribosomes. TMV RNA stimulates the formation of a product which upon hydrolysis with trypsin yields a relatively small number of peptides, as has been observed previously with extracts of *E. coli*.[2, 5] The fingerprint pattern does not correspond to that of the TMV coat protein. Failure of TMV RNA to induce the synthesis of its coat protein may therefore be an intrinsic property of the RNA itself, and not involve deficiencies in one or the other extract. Nevertheless, the TMV product differs from the f2 product, showing that the specificity of protein synthesis by chloroplast ribosomes is under the direction of the stimulating template RNA.

Our experiments do not allow us to say that f2 coat protein is the sole product made in Euglena extracts stimulated by f2 RNA. No purification of the coat protein from the extracts has been attempted, and the electropherograms show some radioactivity in material other than the known tryptic peptides of the coat protein. Similarly, electropherograms of tryptic hydrolysates of unfractionated *E. coli* extracts stimulated by f2 RNA also reveal some unidentifiable material.

Neither quantitative nor qualitative comparison of the unidentified material in stimulated extracts from the two species has been undertaken.

The formation of f2 coat protein in extracts of *Euglena gracilis* can hardly be attributed to a fortuitous similarity between the components of protein synthesis of Euglena and of *E. coli*. The two organisms are phylogenetically remote. The nucleotide composition of the chloroplast ribosomes, with an adenine to cytosine ratio of 1.5,[17] is quite different from that of *E. coli* ribosomes (adenine to cytosine ratio of 1.1, see ref. 18).

The participation *in vitro* by extracts of one species in the correct interpretation of the information encoded in the template RNA of another species is compatible with the report that the alkaline phosphatase from *E. coli* can be produced by *Serratia marcescens* when infected with an episome bearing the gene for the enzyme;[19] it is also compatible with recent reports[20, 21] that animal viruses can be produced in *Bacillus subtilis* transformed with viral DNA. Correct translation of the information in nucleic acid into appropriate sequences of amino acids by distant species demonstrates the universality of the genetic code. However, correct translation does not necessarily indicate that the ribosomes and the soluble components, responsible for amino acid activation or for peptide bond formation itself, are compatible between species. Preliminary results show that the ribosomes and the soluble components must come from the same source. When the soluble factors from *Euglena gracilis* were replaced by the supernatant fraction from *E. coli*, RNA from f2 and from other sources failed to stimulate the chloroplast ribosomes.[22] Despite the fact that many or all of the soluble components and the ribosomes are incompatible between distant species,[6, 7] the primary structure of the protein synthesized is determined by the template RNA.

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*IRREVERSIBLE ELECTROCHEMICAL PRECIPITATION OF MAMMALIAN PLATELETS AND INTRAVASCULAR THROMBOSIS**

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Abramson[1] first indicated the electrophoretic mobility of mammalian blood cells in 1928, and suggested the possible relationship between cell charge, electroosmosis, and blood clotting. Since then, Sawyer and Pate,[2] and Sawyer, Deutch, and Pate[3] have indicated the contrasting thrombogenic and antithrombotic characteristics of oppositely polarized electric currents on normal blood vessels. There has been considerable speculation concerning the possible nature and characteristics of the surface charge of blood cells with particular reference to platelets, and the possible significance of this charge in the development of intravascular thrombosis.[1, 3]

Sawyer, Brattain, and Boddy[4] have recently determined that anticoagulant-free human erythrocytes and leukocytes suspended in Krebs solution at a pH of 7.4 precipitate reversibly on a platinum electrode at an electrode potential (hereafter referred to as V_H) of approximately $+0.33 \pm 0.05$ volts with reference to the normal hydrogen electrode.

These experiments have now been extended to the measurement of the precipitation potential of human and canine platelets from platelet-rich plasma drawn into either heparin or 10 per cent acid citrate dextrose solution (ACD, supplied by Abbott Laboratories). In addition, the precipitation potentials of erythrocytes and leukocytes in both ACD and heparin solutions were measured as controls.

Materials and Methods.—The apparatus previously described by Sawyer, Brattain, and Boddy[4] was used. It was modified to include a microammeter for measuring the current flow across the electrode solution interface during the experiment (Fig. 1).

Platelet preparation: Twenty to 50 ml of canine or human blood were drawn into a clean uncoated glass syringe containing preselected amounts of ACD solution or heparin. The blood was centrifuged at 800 rpm for 15 min at 0°C producing a supernatant plasma with a high concentration of platelets determined by carrying out a platelet count. The platelet-rich plasma was used within 2 hr of preparation.