DNA-DEPENDENT RNA-DIRECTED PROTEIN SYNTHESIS IN VITRO, II. SYNTHESIS OF A φX-174 COAT PROTEIN COMPONENT

By David H. Gelfand and Masaki Hayashi

DEPARTMENT OF BIOLOGY, REVELLE COLLEGE, UNIVERSITY OF CALIFORNIA (SAN DIEGO), LA JOLLA

Communicated by Clifford Grobstein, March 10, 1969

Abstract.—We present evidence to show that the primary protein product of an *in vitro* DNA-dependent RNA-directed protein-synthesizing system primed by *φX*-174 replicative form DNA comigrates in a gel electrophoresis system with a phage structural component. This protein component is precipitable by antiserum to purified phage.

Introduction.—A previous publication[1] from this laboratory described the preparation of an *in vitro* DNA-dependent RNA-directed protein-synthesizing system (the coupled system) using the replicative form of φX-174 DNA (RF DNA) as a template. With DNA-RNA molecular hybridization techniques, it was demonstrated that asymmetric transcription of the entire genome was obtained under conditions of concomitant RNA and protein synthesis. In order to characterize the protein product of this *in vitro* system, a gel electrophoresis assay was developed[2] for the detection of φX-174 specific proteins synthesized *in vivo*. With this technique we were able to identify six different proteins synthesized following infection with amber mutants of φX-174.

We now wish to report that several of the *in vivo* proteins may be synthesized *in vitro* and that the major *in vitro* protein product co-electrophoresed with one of the φX-174 specific *in vivo* labeled proteins and that this *in vitro* protein is precipitable by φX-174 antiserum.

Materials and Methods.—Phage, bacteria, media, buffers, radioactive materials, preparation and description of the *in vitro* coupled system, preparation and isolation of φX-174 specific *in vivo* synthesized proteins, and gel electrophoresis have all been previously described.[1, 2] Calf thymus DNA was purchased from Worthington Biochemicals.

Immunological assay: φX-174 antiserum was prepared by immunizing rabbits with purified wild-type φX-174. S^{35} φX-174 phage, the *in vitro* protein product of a calf thymus DNA-primed coupled system or a φX-174 21*S* replicative form DNA-primed coupled system, was incubated with an appropriate amount of antiserum in 0.15 *M* NaCl, 0.01 *M* Tris-HCl, pH 7.4 (tris-salt buffer) at 37°C, for 30 min and then overnight at 0°C. After the addition of cold φX-174 as carrier, the mixtures were incubated at 37°C for 30 min and at 0°C for 1 hr. The precipitates were centrifuged, washed twice with cold tris-salt buffer, and resuspended in 0.3 ml sample buffer. Aliquots were either subjected to gel electrophoresis or heated at 90°C in 7% perchloric acid, then neutralized with NaOH, precipitated with cold 10% trichloroacetic acid, filtered, washed, dried, and counted (see Table 1).

Results.—A previous communication[2] identified the protein components synthesized after infection with amber mutants in groups *A* to *F* under our conditions of electrophoresis. Figure 1 shows a typical electrophoretic pattern obtained when H^3-labeled φX-174 wild-type *in vivo* protein is co-electrophoresed with RF DNA primed ^{14}C-labeled *in vitro* synthesized protein. While *E* protein is probably synthesized and *A* and/or *B*, and *C* may be synthesized,

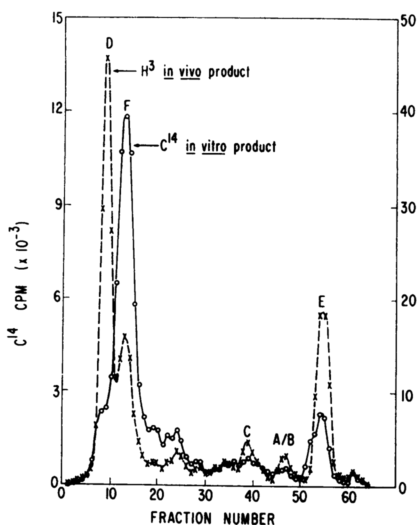

FIG. 1.—Acrylamide gel electrophoresis of ^{14}C *in vitro* and ^{3}H *in vivo* protein product. *In vitro* protein was labeled from 0–30 min, extracted with phenol, dialyzed against gel sample buffer, and electrophoresed with ^{3}H-labeled protein extracted from ϕX-174 wild-type infected cells. Gels were run from cathode to anode (right to left) and sliced from the anode end. ^{14}C *in vitro* (O—O), ^{3}H *in vivo* (×—×).

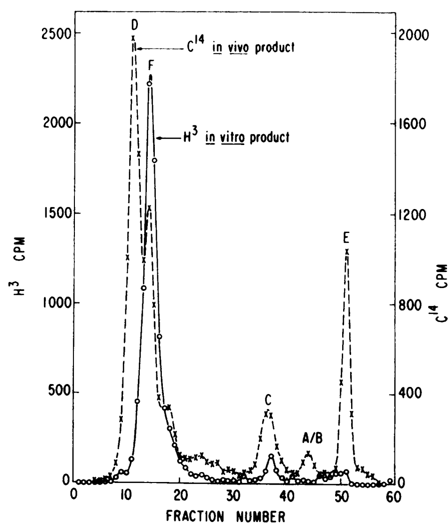

FIG. 2.—Acrylamide gel electrophoresis of antibody precipitated ^{3}H *in vitro* product and ^{14}C *in vivo* product. *In vitro* protein was pulse-labeled with ^{3}H-leucine for 0–30 min and chased with a 100-fold excess of cold leucine for 30–60 min. ϕX-174 anti-serum was added and the sample was treated as described in *Materials and Methods*. ^{3}H *in vitro*, antibody precipitable product (O—O), ^{14}C *in vivo* product (×—×).

the majority (55%) of the *in vitro* synthesized protein appears in a peak that comigrates with *in vivo* synthesized *F* protein.

The *F* protein has been shown to be a phage spike component and is responsible for serum blocking power.[3] Table 1 indicates that our ϕX-174 antiserum precipitated 95 per cent of S^{35}-labeled ϕX-174 and about 40 per cent of the RF DNA-primed coupled system protein product synthesized *in vitro* but only about 7 per cent of the calf thymus DNA-primed coupled system protein product synthesized *in vitro*. Figure 2 shows the electrophoretic pattern obtained when H^3-labeled *in vitro* protein product of a RF DNA-primed coupled system that has been purified by precipitation with ϕX-174 antiserum is co-electrophoresed with ^{14}C-labeled ϕX-174 wild-type *in vivo* synthesized protein. It can be seen that

TABLE 1. *Precipitability of protein with anti-ϕX-174-serum.*

Sample	Total counts in antibody precipitate (%
S^{35} ϕX-174	95.2
ϕX-174 21S replicative form DNA-primed coupled system	39.3
Calf thymus DNA-primed coupled system	7.1

ϕX-174 antiserum was added to either S^{35} ϕX-174 phage, RF DNA-primed coupled system protein product, or calf thymus DNA-primed coupled system protein product (see *Materials and Methods*). Data represents the per cent of initial sample counts recovered in the antibody precipitate.

most (80%) of the antibody precipitable counts comigrate with the *F* protein synthesized *in vivo*.

Discussion.—A previous communication[2] assigned the following functions to each of our seven complementation groups: *A*, phage protein; *B*, phage protein; *C*, RF DNA duplication; *D*, single-strand DNA synthesis; *E*, capsid protein; *F*, "spike" protein; *G*, lysis. From the results presented in this paper, it is clear that the coupled system previously reported[1] preferentially synthesizes a protein which comigrates with the *F* protein. Furthermore, antibody precipitation of the *in vitro* product indicates that the precipitated protein also comigrates with *F*, the phage component identified as being responsible for serum blocking power.[3] While confirmation of total identity of our major *in vitro* product and the *F* protein will have to wait for fine peptide analysis or determination of amino acid sequence, it seems that if initiation, termination factors are required for synthesis of "*F*" protein *in vitro*, our coupled system probably contains these factors.

It is clear that the system provides many possibilities for studying the regulation of protein synthesis *in vitro*. The various effectors for the preferential synthesis of "*F*" protein *in vitro*, and increasing the proportion of other phage proteins are under investigation.

This research was funded through National Science Foundation grant GB-6259 and the Public Health Service grant GM-12934. One of us (D. H. G.) is the recipient of a Public Health Service predoctoral traineeship grant GM-00702.

[1] Bryan, R. N., M. Sugiura, and M. Hayashi, these PROCEEDINGS, 62, 483 (1969).

[2] Gelfand, D. H., and M. Hayashi, *J. Mol. Biol.*, submitted.

[3] Sinsheimer, R. L., in *Progress in Nucleic Acid Research in Molecular Biology*, ed. J. N. Davidson and W. E. Cohn (New York: Academic Press, 1968) vol. 7, p. 1153.