

pupae and pupae of the house fly, *Musca domestica* L., which are found commonly in breeding sites with the stable fly (10).

Therefore, in 1972, we established a colony of *M. raptor* in the laboratory to determine whether the JH analogs would affect parasitic development and reproductivity.

The test procedure was as follows. New stable fly pupae, still opaque white, were treated topically with 10 µg of the JH analogs in 1 µl of acetone. Then 25 pupae were placed in holding cages, and six females and four males of *M. raptor* (3 to 4 days old) were immediately placed in the cage with the treated pupae. The holding temperature was 27° ± 1°C, and the relative humidity was 90 percent. The controls were treated and untreated pupae that were not exposed to the parasite and untreated pupae that were exposed. At 9 days posttreatment, the treated pupae that had not been exposed to the parasite were evaluated by determining the number of adults that eclosed and the number of pupal-adultoid intermediates within the puparia. At 20 days posttreatment, we recorded the number of parasites that emerged from the treated and untreated pupae exposed to the parasite. Also, we dissected these pupae to determine whether additional parasites were present as the result of the diapause that will occur in *M. raptor* (11). This experiment was replicated five times.

Finally, adult *M. raptor* that emerged from the treated and untreated pupae were placed separately with untreated pupae and allowed to feed and oviposit for 20 days. The numbers of parasites that emerged from these two groups were then compared to determine the effects on the reproductivity of parasites that had developed in stable fly pupae treated with the JH analog.

Table 1 shows that when the two JH analogs were applied to stable fly pupae, the development of *M. raptor* was not affected although the dose administered was 1000 times more than required to obtain a pupal-adultoid intermediate in the stable fly (5). No pupae eclosed that were exposed to *M. raptor*, the JH analogs, or both. Adult stable flies emerged from untreated pupae at 7 days; however, when the pupae treated with the analogs were dissected, pupal-adultoid intermediates were found within the puparia. Since the JH analogs do not

Table 2. Development of progeny of adult *M. raptor* that developed in stable fly pupae treated with the two juvenile hormone analogs (25 pupae per test; progeny developed in untreated pupae). Values are numbers of *M. raptor* F₁ progeny eclosing when P₁ developed in stable fly pupae.

Treated with JH analog 1	Treated with JH analog 2	Untreated
19	18	16
11	15	13
13	11	13
16	18	15
17	17	16

immediately kill the stable fly pupae (the metamorphosing pupae live 6 to 7 days before death occurs), *M. raptor* has time to develop. Thus, at 7 days the parasite was in the pupal stage within the stable fly puparium and the adult parasites then emerged 7 to 15 days later.

Table 2 shows that the reproductive ability of *M. raptor* adults that developed within the stable fly pupae treated with the JH analogs was not affected. These adults oviposited viable eggs in untreated stable fly pupae, and the F₁ generation developed and eclosed.

The significance of the normal development of the parasite in the pupae treated with the JH analogs is that the chemicals were selective against the stable fly pupae and not *M. raptor*. Even the reproductivity of this beneficial parasite was not affected. Thus, a new approach for integrated control of insects has been demonstrated, namely, the use of a chemical JH

analog in conjunction with a parasitic wasp, *M. raptor*, for control of the hematophagous stable fly.

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8 September 1972

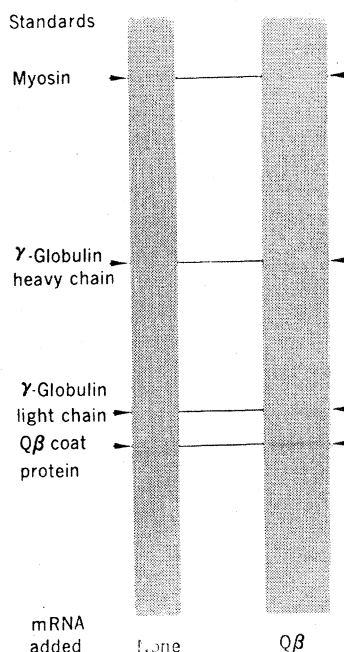
Translation of Bacteriophage Q β Messenger RNA in a Murine Krebs 2 Ascites Tumor Cell-Free System

Abstract. Q β is a small bacterial virus whose three genes are encoded in a single-stranded molecule of RNA. This RNA serves directly as the Q β message. Here we describe conditions under which RNA corresponding to the coat cistron of this bacterial virus is translated in a system derived from mammalian cells. Translation of the bacterial virus messenger RNA is less effective than that of mammalian globin messenger RNA, but is somewhat enhanced by mild alkali treatment of the messenger. The synthesized product when subjected to electrophoresis migrates with authentic Q β coat protein and yields tryptic peptides that correspond to those derived from the Q β coat protein.

Universality is one of the striking features of the genetic code implying that there are strong selective pressures which maintain the code intact among species that diverged millions of years ago (1). Thus, the core word for phen-

ylalanine is the same in both bacteria and mammals. Nevertheless, the early studies of Nirenberg and his co-workers (2) suggested that there were subtle differences among the code words used preferentially by different species. Other

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the Q β mRNA-directed product of the Krebs 2 ascites tumor cell-free system. Components of the Krebs 2 ascites cell-free system (5) and Q β mRNA (8) were prepared as described. Each 0.06-ml reaction mixture contained 30 mM tris-HCl (pH 7.5), 3.6 mM magnesium acetate, 53 mM KCl, 7 mM 2-mercaptoethanol, 1 mM adenosine triphosphate, 0.1 mM guanosine triphosphate, 0.6 mM cytidine triphosphate, 10 mM creatine phosphate, 8 μ g of creatine kinase, 40 μ M (each) non-radioactive amino acids, 5 μ M radioactive amino acids ($[^{14}\text{C}]$ leucine, $[^{14}\text{C}]$ valine, $[^{14}\text{C}]$ lysine, $[^{14}\text{C}]$ serine, and $[^{14}\text{C}]$ alanine), 0.2 A₂₆₀ unit of rabbit tRNA, 0.12 A₂₆₀ unit of ribosomes, 0.12 mg of protein in ribosome-free supernatant, and 30 μ g of Q β RNA, as indicated. The reaction mixture was incubated at 37°C for 150 minutes. Samples were prepared, subjected to the sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and exposed to x-ray film as described (11).



studies have suggested that the translation mechanism is not generally available to all messenger RNA's (mRNA) regardless of species and that, in fact, there is translational discrimination among various mRNA's (3). These studies have focused on the involvement of initiation in this restrictive process and further suggest that this mechanism discriminates between different classes of mRNA from the same organism.

Despite these suggestions, several

mRNA's have been translated in vitro in systems that cross species barriers (4). While these results may have been obtained in spite of subtle translational control mechanisms, the availability of RNA phage carrying a variety of amber mutations that might prove useful in identifying mammalian suppressors, encouraged us to try to translate this mRNA in a transfer RNA-dependent, mammalian cell-free system (5). Our early experiments indicated that RNA derived from the bacteriophage Q β

would direct the synthesis of protein in this mammalian system (6). These results have been confirmed (7), but in neither case was the protein product shown to correspond to an authentic phage protein. Here we show that, under appropriate conditions, the cistron coding for the bacteriophage Q β coat protein is translated to a polypeptide chain that has the same molecular weight as authentic Q β coat protein and yields tryptic peptides corresponding to those derived from the authentic bacteriophage protein.

Our mammalian cell-free system was derived from Krebs 2 ascites tumor cells already described (5). This system is particularly convenient in that it responds to the addition of very small amounts of mRNA and is dependent on the addition of transfer RNA (tRNA) as well. The growth, isolation, and extraction of RNA from the bacteriophage Q β have been described (8), as has the procedure used in the preparation of rabbit reticulocyte mRNA (9). The conditions under which the Q β mRNA was translated were determined by selecting the magnesium, potassium, and Q β mRNA concentrations that gave optimum incorporation of radioactive amino acids (Table 1).

As might be expected, the bacteriophage RNA is less active as mRNA in the mammalian cell-free system than is rabbit globin (Table 1). Approximately 30 μ g of native Q β RNA

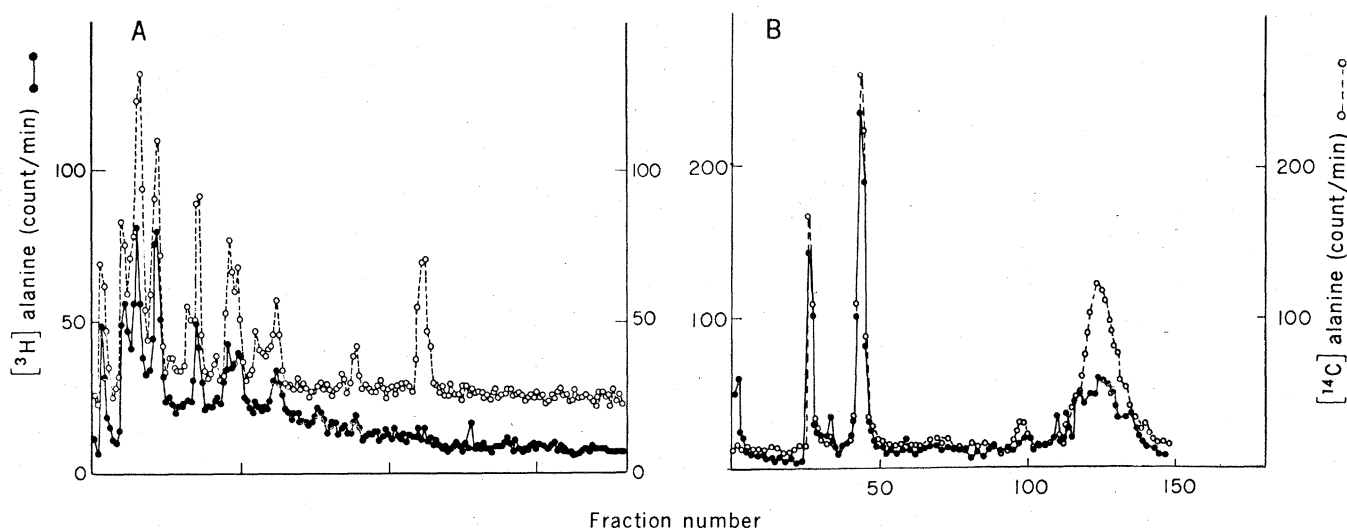


Fig. 2. Ion exchange chromatography of $[^{14}\text{C}]$ - and $[^3\text{H}]$ alanine-labeled tryptic peptides from authentic Q β coat protein and from polypeptide obtained from Q β mRNA-directed synthesis. Components and conditions necessary for synthesized products were as described in the legend to Fig. 1, except that $[^3\text{H}]$ alanine and 19 unlabeled amino acids were used. Coat protein labeled with $[^{14}\text{C}]$ alanine was obtained from $[^{14}\text{C}]$ alanine-labeled Q β bacteriophage. Both the naturally occurring and the synthesized products were prepared, a derivative was made, and the derivative was digested with trypsin and applied to the chromatograph column as described (12). (A) Chromatograph on Technicon PI ion exchange resin. One-tenth of each preparation was taken for counting from fractions 1 to 7 (nonabsorbed). (B) Chromatograph on Dowex-1 of fractions 1 to 7 from the Technicon PI column. Radioactivities of ^3H and ^{14}C were determined simultaneously in each dried sample by liquid scintillation counting. $[^3\text{H}]$ Alanine is in the synthetic product; $[^{14}\text{C}]$ alanine is in the authentic Q β coat.

directed a three- to fourfold increase in the incorporation of [³H]leucine as compared to the value obtained in the absence of mRNA. Treating the RNA with formaldehyde to reduce its ordered structure or, briefly, with mild alkali to partially degrade the mRNA resulted, respectively, in a slight loss and a slight enhancement of activity. In contrast, a much smaller amount of rabbit reticulocyte polysomal RNA (6 µg) induces a greater than 30-fold increase in the incorporation of [³H]leucine. Rabbit globin and Qβ coat protein contain comparable amounts of leucine.

Although it is evident that the phage RNA is a less efficient template for protein synthesis in this cell-free system, the product, as analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, consists of a single polypeptide which comigrates with authentic Qβ coat protein (Fig. 1). The synthesized product yields tryptic peptides that correspond to each of the major Qβ coat protein tryptic peptides (Fig. 2), and also yields one prominent peak containing alanine (Fig. 2A, fractions 109–114) and two smaller peaks (52–53, 55–56) that are absent or present to only a small extent in the coat protein. These extra peptides may be related to the processing of an initial, Qβ coat peptide (fMet-Ala-; that is, formylmethionylalanine) or possibly to the partial translation of one of the noncoat cistrons. It is apparent, however, that, as in bacterial cell-free systems, the Qβ coat protein is translated *in vitro* in marked preference to the other two Qβ cistrons (8).

The fact that the Qβ coat cistron is translated in a mammalian cell-free system emphasizes the universality of the translation process. At the same time, it does not rule out subtle translational discriminatory mechanisms that may operate *in vivo*, but which are overcome by selecting conditions favorable for the translation of a specific, heterologous mRNA. This observation does have rather important practical implications, however, because it provides an operational connection between a mammalian cell-free system and a bacterial virus mRNA in which a variety of suppressible termination mutants are available. Thus, the system, in combination with an appropriate amber mutation in the Qβ coat protein, should provide a technique for surveying tRNA's from mutagenized lines of mammalian cells for their ability to suppress amber mutations. Recent ex-

Table 1. Protein synthesis in response to bacteriophage Qβ mRNA. Each reaction mixture contained the components, and was incubated under conditions described in the legend to Fig. 1, except that [³H]leucine plus 19 unlabeled amino acids were used. Where indicated, 6 µg of rabbit reticulocyte polysomal RNA was added. The reaction mixtures were precipitated with trichloroacetic acid, and the precipitate was then assayed. The modified Qβ RNA's were incubated in 0.01M tris, pH 7.2, containing either 3 percent formaldehyde or in 0.01M tris, pH 9.0, at 37°C for 30 minutes and dialyzed against H₂O.

Addition	[³ H]Leucine incorporated (pmole)
None	0.24
Qβ RNA	0.87
Qβ RNA, formaldehyde treated	0.56
Qβ RNA, pH 9.0 treated	1.27
Rabbit reticulocyte polysomal RNA	8.69

periments involving the use of Qβ and R17 bacteriophage mRNA's in a variety of mammalian cell-free systems suggest that this approach will be generally applicable (10). The identification of a suppressor-containing mammalian cell line would be of obvious value in studies involving animal viruses and cultured cells.

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8 September 1972

Genital Sensory Field: Enlargement by Estrogen Treatment in Female Rats

Abstract. *Recordings of neuronal activity in the pudendal, genitofemoral, and pelvic nerves indicate that the sensory fields of these three nerves are the perineum, the caudal abdomen, and the vagino-cervical area and rectum, respectively. The sensory field of the pudendal nerve was significantly larger in estrogen-treated ovariectomized female rats than in uninjected controls. This effect of estrogen was not mediated by pudendal efferents.*

Recent analyses of hormonal influences on behavior and the nervous system have emphasized the effects of hormones directly on the brain (1) and spinal cord (2). However, hormones may also influence behavior by altering peripheral sensory mechanisms. In humans, acuity of the senses of touch, taste, and olfaction are altered by hormones (3), and on the basis of behavioral responses in animals, it has been proposed that hormones may act at the periphery to alter sensory input

(4). However, there appears to be no previous neurophysiological evidence that hormones change peripheral neural activity. In one attempt to demonstrate an androgenic influence on penile sensitivity, recordings from the pudendal nerve in cats revealed no significant effect of androgen (5).

Perineal stimulation in female rats occurs prior to intromission, during copulatory thrusting by the male. This stimulation is likely to play a major role in facilitating the mating stance