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Bacteriophage f1 infection of *Escherichia coli*: Identification and possible processing of f1-specific mRNAs *in vivo*

(protein synthesis/gene V and VIII proteins/rifampicin)

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ABSTRACT [^{3}H]Uracil-pulse-labeled RNA from *Escherichia coli* infected with f1 bacteriophage was fractionated on polyacrylamide gels containing urea. Eight phage-specific RNA species were present with approximate lengths ranging from 2100 to 400 nucleotides. The amount of the seven largest species was increased when the infected bacteria were incubated at 42°C. When the RNA was isolated and used as message in an *in vitro* protein-synthesizing system, most of the RNA species appeared to direct the synthesis of the phage gene VIII protein. The six largest species also directed the synthesis of the phage gene V protein. Some of the labeled smaller RNA species increased in amount after addition of rifampicin, suggesting that they may have resulted from cleavage of larger RNA species. These particular smaller RNA species also were present in infected bacteria containing a mutant RNase III. The data are discussed in terms of the regulation of synthesis of the phage-specific proteins.

The closely related filamentous bacteriophage f1, fd, and M13 contain a single-stranded DNA that codes for approximately eight genes (1, 2). *Escherichia coli* infected with these phage continue to grow and divide, while newly synthesized phage are extruded through the membrane. The identification of most phage products has been hampered by this continuation of host growth. However, products of the phage genes V and VIII (coat protein) are synthesized in large quantities and can be easily identified (3, 4). The two proteins are also the major products of coupled transcription–translation directed *in vitro* by replicative form DNA (5, 6). These findings suggest that there is some regulatory mechanism operating both *in vivo* and *in vitro* that allows this differential expression of phage products.

Such a regulatory process may be operating partially at the level of transcription. Studies *in vitro* have shown that a small number of discrete transcripts are synthesized; initiation is from various promoters, while termination appears to occur at a unique site (7–11). Due to their location in the genome (12), the gene V and VIII regions are immediately proximal to the termination site; thus, most mRNAs *in vitro* would contain the coding information for these genes.

In order to examine the basis for the high level of expression of the gene V and VIII proteins *in vivo*, it is necessary to characterize phage-specific mRNA found in infected bacteria. We report here the identification of a number of phage-specific mRNAs and show that several of them can direct the synthesis of the gene V and VIII proteins. Some of the species appear to correspond to those found *in vitro*, while others may have arisen as a result of processing from larger RNA molecules. These results are discussed in terms of the regulation of synthesis of the phage-specific proteins.

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MATERIALS AND METHODS

E. coli K38, a nonsuppressing strain, and K37, a serine-inserting suppressing strain, were used (13). BL214, an RNase III$^-$ strain (14), was obtained from W. Studier. Bacteriophage f1, f1 amber mutants, and media have been described (15). f1-R111, a gene V temperature-sensitive mutant, was received from P. Model. The methods for the labeling, isolation, and identification of phage-specific RNA and protein products are described in the figure legends.

RESULTS

RNA of uninfected bacteria and bacteria infected with f1 wild-type or R111 mutant phage was labeled for 60 sec with [^{3}H]uracil, extracted, and separated by electrophoresis on polyacrylamide gels containing 7 M urea (16). Examination of the resulting fluorogram (Fig. 1) showed that infected bacteria (lanes b and d) produce an intense band (H) and a number of faint bands (A–G), that appeared to have no counterparts in, or were increased over, the uninfected sample (lane a). Under these conditions, there was little radioactivity in bands A–G, sometimes making it difficult to detect them. However, when the infected bacteria were incubated at 42°C (lane c), the intensity of all the bands except band H increased (data not shown). Similar labeling at 42°C (nonpermissive temperature) of bacteria infected with f1-R111 (lane e) showed an even further increase in the amounts of radioactivity in bands A–G. In both cases, high temperature resulted only in increased amounts of these phage-specific bands and not in the appearance of more bands. Since R111-infected bacteria at 42°C produced the highest level of these phage-specific bands, these infected cells were used in all subsequent experiments. All of the material visualized in the fluorograms represents [^{3}H]uracil present in RNA, since the label is greater than 99% solubilized in the presence of 1 M KOH, at 37°C for 2 hr, conditions which do not affect DNA. When these same gels in Fig. 1 were stained with ethidium bromide, there were many fluorescent bands that did not correspond to the labeled species present in the radioactive fluorogram. Thus, the [^{3}H]uracil-labeled material represents RNA species that are newly synthesized following infection. Comparison of the mobility of these labeled species with specific RNA standards (Fig. 1) showed that bands A–H correspond to RNAs of approximately (±SD) 2452(±64), 1464(±179), 1190(±78), 1078(±44), 1027(±39), 966(±38), 486(±33), and 412(±36) nucleotides, respectively.

La Farina and Model (19) have shown that RNA extracted from bacteria infected with f1 bacteriophage can direct the synthesis *in vitro* of the gene V and gene VIII proteins and, at a much lower level, the gene III protein. We observed the same results by using the RNA extracted from infected cells grown at 35°C or 42°C. Therefore, the *in vitro* protein-synthesizing

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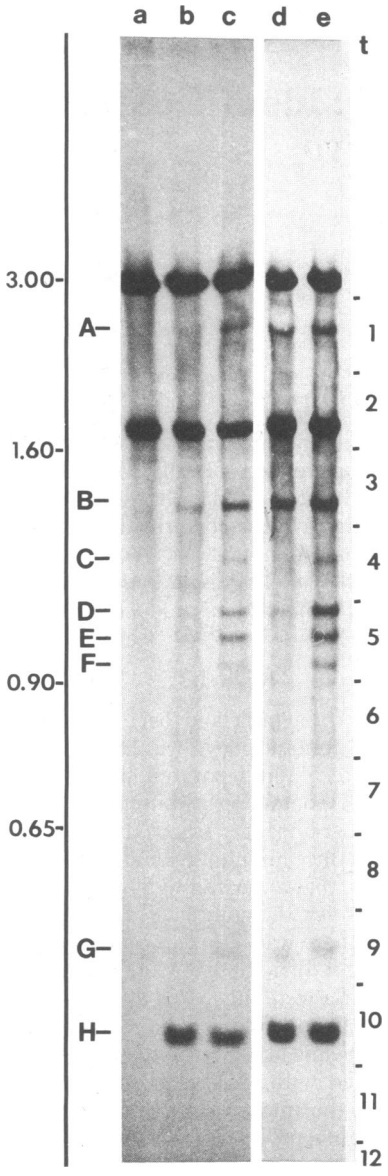

FIG. 1. Electrophoresis of RNA in an acrylamide/7 M urea gel. *E. coli* K38 bacteria were grown at 35°C in modified MTPA medium (15) to a cell density of 1.5×10^8 bacteria per ml. Cultures were infected with f1 wild-type or f1-R111 (ts gene 5) phage at a multiplicity of infection of 200; after 11 min, half of each culture was transferred to 42°C. Six minutes later, [5-^{3}H]uracil (3 μCi/ml, 30 Ci/mmol, 1 Ci = 3.7×10^{10} Bq; New England Nuclear) was added; after 1 min, a 5-ml sample was quickly chilled and centrifuged. The bacteria were resuspended in 1.5 ml of 10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.5% sodium dodecyl sulfate and briefly incubated at 60°C until clear. The RNA was extracted twice with phenol, precipitated and washed with ethanol, and dried under N_2. RNA slab gels ($14.5 \times 30 \times 0.3$ cm) were prepared according to Spralding *et al.* (16) 24 hr in advance and contained a 2.5–5.25% acrylamide gradient and 7 M urea. Samples were incubated at 65°C for 5 min prior to layering and electrophoresis was at 140 V for 16 hr. Gels were then prepared for fluorography and exposed to prefogged Kodak X-Omat x-ray film (17, 18). This figure is a fluorograph of the top two-thirds of the gel (t is the top). Putative phage-specific bands are designated A–H. RNA was eluted from regions 1–12 for the protein synthesis described in Fig. 2. The numbers on the left are the approximate lengths of unlabeled standards present in the same gel (nucleotides $\times 10^{-3}$): *E. coli* rRNA (3.0 and 1.6), brome mosaic virus RNA (0.9), and globin mRNA (0.65). Lanes: a, RNA from uninfected bacteria at 42°C; b, f1 (wild type) infected at 35°C; c, f1 infected at 42°C; d, f1 R111 (ts5) infected at 35°C; and e, f1 R111 infected at 42°C. Equal amounts of radioactivity were added to each slot.

system was used to determine which of the fractionated species in Fig. 1 were phage-specific RNA. The gel containing the uninfected and infected RNA (Fig. 1) was fractionated into 12 sections; RNA was eluted and used to direct the synthesis of proteins *in vitro*. As shown in Fig. 2*B*, the RNA from regions 9 and 10, containing bands G and H, synthesized a protein that migrated at the position expected for gene VIII protein. RNA from the regions 3–5, containing bands B–F, directed the synthesis of proteins of the sizes of the gene V and VIII products. It is possible that RNA from region 1, containing band A, also directs the synthesis of these two proteins, although this is not clear. Sometimes a faint band migrating at the position expected for the putative gene VII product (23) could be detected with RNA from regions 3–5. Although it is not visible at the exposure of the fluorogram shown in Fig. 2, it is visible in Fig. 4 (slot a). Gene VII is located between gene V and gene VIII of the DNA (24); thus, any message coding for both gene V and VIII proteins should contain the information for gene VII. These proteins were not synthesized when fractionated RNA from uninfected bacteria was similarly isolated (Fig. 2*A*). Since this analysis did not show that RNA from regions containing individual bands could synthesize the presumptive gene V and VIII proteins, the RNA was eluted from narrower cuts of the central part of the gel and subjected to the same analysis (Fig. 3). In this case, the RNA from regions containing only bands B, C, D, E, and F all directed the synthesis of these two proteins.

To determine whether two of the proteins in question were the gene V and VIII products, we extracted RNA from nonpermissive cells infected with gene V or VIII amber mutant and wild-type phage. After fractionation of RNA by gel electrophoresis, the two regions containing the most active mRNA were analyzed in the *in vitro* protein-synthesizing system. As shown in Fig. 4, region 5 RNA (bands D–F, Fig. 1) from bacteria infected with wild-type phage produced the presumptive gene V, VII, and VIII products (lane a), and region 10 RNA (band H) synthesized the gene VIII protein (lane b). However, only gene V protein and no gene VIII protein was synthesized with region 5 RNA from bacteria infected with gene VIII amber mutant (lanes c and d), and no gene VIII protein was made from region 10 RNA (lanes e and f). When RNA from bacteria infected with gene V amber mutant was used, the material from region 5, containing bands D, E, and F, did not direct the synthesis of any protein migrating at the position expected for gene V protein (lanes g and h). The amount of gene VIII protein also was decreased, indicating a possible translational polarity effect due to secondary structure of the RNA, as might be expected since the information for the gene V protein would be on the 5′ side of any polygenic message coding for both gene V and VIII protein (23). RNA from the region containing band H did code for the gene VIII protein (lanes i and j).

These data suggest that the [^{3}H]uracil-labeled material in bands A–H (Fig. 1) represents *in vivo* f1 phage-specific RNA transcripts. Since there were more labeled RNA species than would have been expected from initiation at the described promoters *in vitro* (7–11), it seemed possible that some *in vivo* species might have resulted from processing. To test this, infected and uninfected bacteria were pulse labeled with [^{3}H]-uracil for 1 min, rifampicin was added to prevent further initiation of message, and the fate of the pulse-labeled mRNA was determined by gel electrophoresis. Under these conditions, synthesis of mRNA stopped approximately 30 sec after the addition of rifampicin. As seen in Fig. 5 (lanes e–h), the radioactivity in bands B, C, and D rapidly disappeared while the radioactivity in bands F and G increased. The intensity of band E remained stable during the first few minutes of the chase and then decreased sharply. Similar results were obtained when the

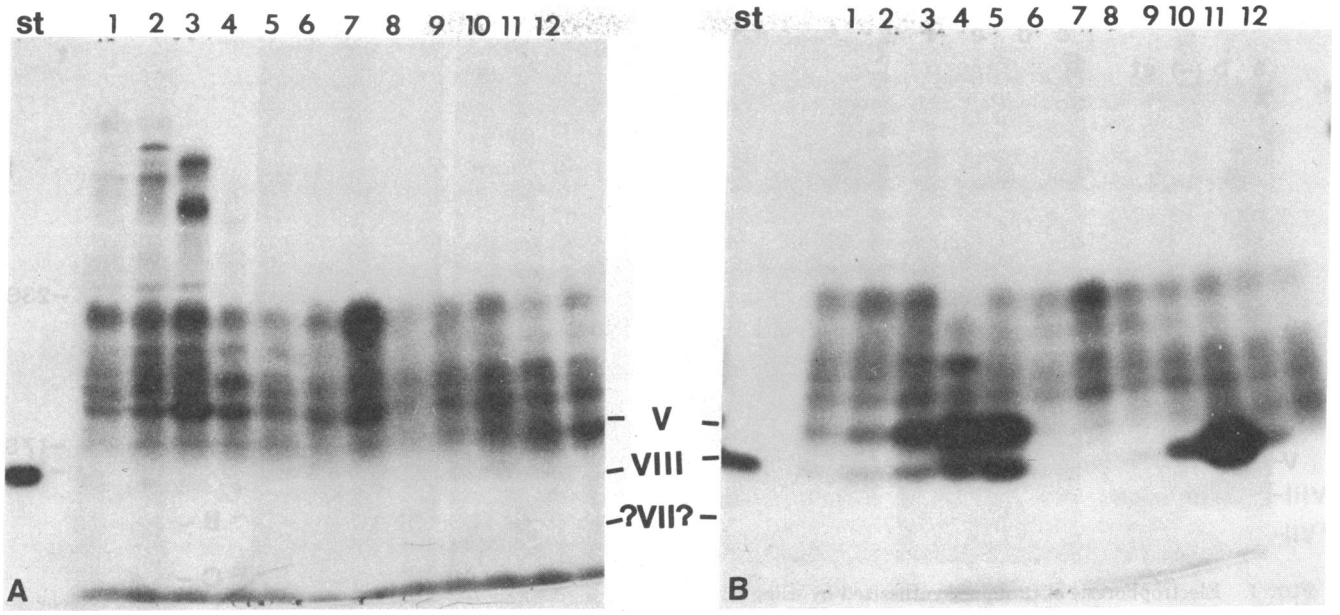

FIG. 2. Electrophoresis of proteins synthesized *in vitro* with acrylamide gel-fractionated RNA. RNA in regions 1–12 (Fig. 1) was eluted (20), further fractionated on Whatman CF-11 cellulose powder (21), and concentrated by ethanol precipitation. The RNA was used to direct an S30 protein synthetic system (13) containing a mixture of ^{14}C-labeled amino acids (New England Nuclear). The products were separated by electrophoresis on 15–22% acrylamide exponential gradient gels containing 8 M urea (22), which were prepared for fluorography as in Fig. 1. The lanes marked st contain labeled coat protein (VIII) from purified phage, and unlabeled gene V protein (V). The size of the gene VII protein has been predicted from the DNA sequence (23). (*A*) Protein synthesis using RNA from uninfected bacteria. (*B*) RNA from R111-infected bacteria. Lanes 1–12 correspond to the regions from the RNA gel in Fig. 1.

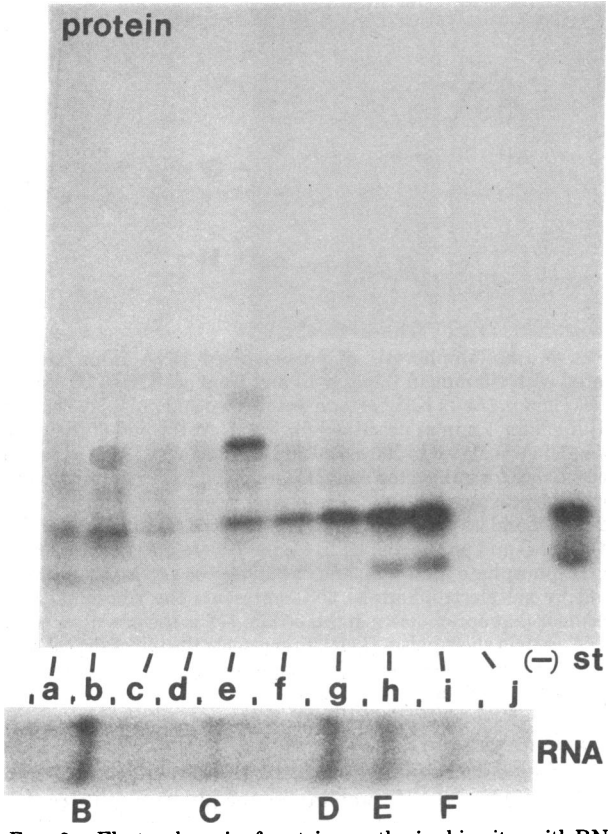

FIG. 3. Electrophoresis of proteins synthesized *in vitro* with RNA from regions 3, 4, and 5 in Fig. 1. By the same techniques as described for Fig. 2, smaller regions of the RNA gel were sliced and the RNA was eluted. (*Lower*) Fluorogram of the section of the RNA gel that was fractionated. (*Upper*) Fluorogram of the gel on which the synthesized proteins were separated. Lanes a–j correspond to regions of the RNA gel as shown. Lane (–) represents the products synthesized in the S30 with no added RNA; st contains labeled gene V and gene VIII proteins. The putative gene VII protein would not be seen on this gel due to the length of the electrophoresis.

RNA was labeled for increasing lengths of time with [^{3}H]uracil or [^{32}P]phosphate and not subjected to rifampicin treatment (data not shown). None of these bands were seen when similarly treated uninfected RNA was examined (lanes a–d). These data suggest that bands E, F, and G may be the result of the cleavage of larger transcripts. The fact that the same labeled species are always seen suggests that the result of this putative processing may be specific. This possible processing appears not to be due to the action of RNase III. When BL214 bacteria (RNase III$^-$ strain, ref. 14) were examined after a 3-min pulse with [^{32}P]-phosphate, the same phage-specific bands were seen (Fig. 5, lane i), while 30S ribosomal RNA precursor was accumulating.

DISCUSSION

A number of investigations have shown that transcription of f1 replicative form DNA *in vitro* gives rise to a series of overlapping RNAs that are initiated at various promoter sites and terminated at a common site just after gene VIII (7–11, 25, 26). These studies predicted that all mRNAs would contain coding sequences for gene VIII, a fact confirmed when it was found that most of these *in vitro* mRNAs directed the synthesis of the gene VIII protein (10). We show in this paper that polyacrylamide gel analysis of RNA from infected bacteria pulse labeled with [^{3}H]uracil revealed a number of mRNA species that were not present in uninfected bacteria treated in the same manner. When these *in vivo* synthesized RNAs were eluted from the gel and used as messengers in an *in vitro* protein-synthesizing system, most of the RNAs directed the synthesis of at least the gene VIII protein. Thus, the same strong termination signal appears to be operative *in vivo* as *in vitro*. Preliminary nucleotide sequence analysis in our laboratory of these RNAs has indicated that bands B, D, E, F, G, and H have the same 3′ terminus as that shown to exist on the 8S mRNA transcribed *in vitro* (25), supporting this hypothesis.

The approximate sizes of some of these *in vivo* species also suggest that a number of *in vitro* identified promoters (8, 9, 11) may be used *in vivo*. In particular, the band H RNA (412 nu-

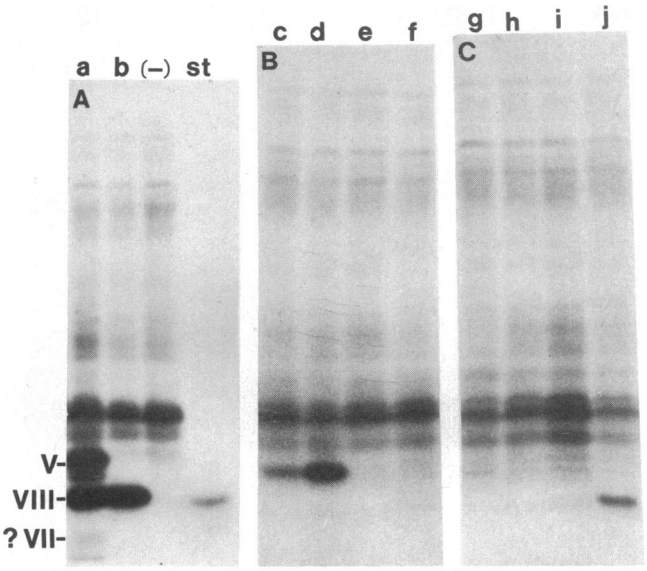

FIG. 4. Electrophoresis of proteins synthesized by using RNA from bacteria infected with wild-type bacteriophage (*A*) and with gene VIII (*B*) and gene V (*C*) amber mutants. The procedure for gel electrophoresis and fluorography was as described for Fig. 2, except that the RNA from only regions 5 and 10 (Fig. 1) was analyzed. Both regions were further subdivided for the analyses in *B* and *C*. (*A*) Lane a, proteins synthesized by region 5 RNA; lane b, region 10 RNA; lane (–), no added RNA; st, labeled gene VIII and unlabeled gene V protein. (*B* and *C*) Lanes c and g, top half of region 5 RNA; lanes d and h, bottom half of region 5 RNA; lanes e and i, top half of region 10 RNA; lanes f and j, bottom half of region 10 RNA. Fluorogram in *A* was overexposed to show the presumptive gene VII protein.

cleotides) is probably the same as the 8S mRNA synthesized *in vitro* with fd DNA and characterized by Sugimoto *et al.* (25). Such an RNA species has already been isolated by a similar gel electrophoresis technique from M13-infected bacteria by Rivera *et al.* (27). They have shown that this RNA has the same termini as the *in vitro* species and can direct the synthesis of the gene VIII protein.

A number of the other labeled RNA species shown in Fig. 1 may correspond to those mRNAs predicted from the studies *in vitro* (11) and the nucleotide sequence of the fd DNA (23). Bands A, B or C, and D or E may correspond to RNAs of 2100, 1140, and 765 nucleotides, respectively, initiated at three identified promoters and terminated at the common termination signal. The fact that these *in vivo* species all contain the gene V and VIII regions and disappear after rifampicin addition is consistent with this view. The sizes, as predicted from the urea gel electrophoresis for these RNAs, are greater than those predicted by the nucleotide sequence (23). Indeed, as indicated by our size for the H mRNA (approximately 412 nucleotides compared to about 370 nucleotides) with the standards in Fig. 1, this gel system appears to give an overestimate of nucleotide lengths. Information on the 5′ sequence of these RNAs is necessary to determine the real nucleotide lengths.

There are more species of RNA in Fig. 1 of approximate length between 1464 and 500 nucleotides and that contain gene VIII than can be accounted for by the known promoters. It is possible that other promoters and terminators may be operative *in vivo* that would result in mRNAs having different termini. Because the RNAs in question all appear to have the same 3′ termini, it is more likely that some or all of them may have arisen as a result of processing from larger phage mRNA species. The fact that some of the larger RNA species rapidly disappear and bands F and G appear after addition of rifampicin is strongly supportive of such an interpretation. It would appear that RNase III may not be responsible for this processing since

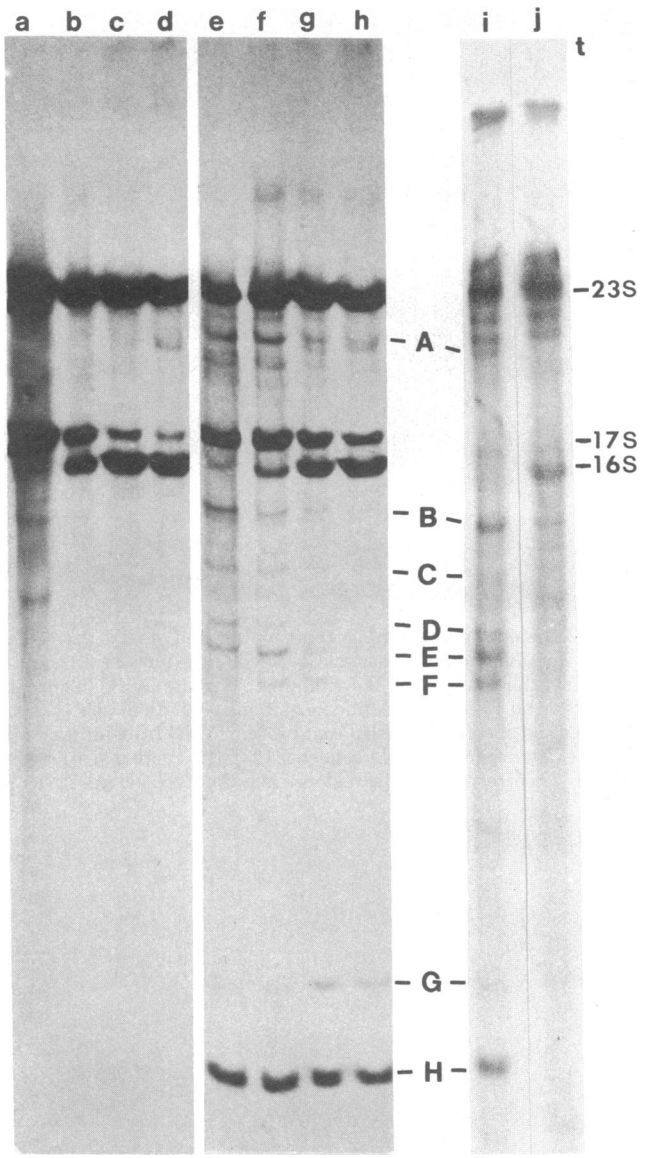

FIG. 5. Electrophoresis of pulse-labeled RNA from bacteria treated with rifampicin (lanes a–h) and from an RNase III$^-$ strain (lanes i and j). (*Left*) K38 bacteria were grown and pulse labeled with [^{3}H]uracil for 1 min as described for Fig. 1. At the end of the pulse, rifampicin was added to 200 μg/ml and samples were taken at various times. Lanes for uninfected and R111-infected bacteria, respectively: a and e, 1-min pulse; b and f, 1 min after rifampicin addition; c and g, 2 min; d and h, 4 min. (*Right*) R111-infected BL214 (RNase III$^-$) (14) (lane i) and uninfected BL214 (lane j) were pulsed with 200 μCi of [^{32}P]phosphate for 3 min, and the RNA was extracted and separated by gel electrophoresis. 30S represents the ribosomal RNA precursor that accumulates in this strain; 17S is the precursor to 16S rRNA, seen in short pulses (*Left*). t represents the top of the gel. Each sample in slots a–h and i and j contains an equal amount of ^{3}H and ^{32}P cpm, respectively.

all these species can be isolated from infected RNase III$^-$ bacteria labeled for 3 min with [^{32}P]phosphate.

The gene V and VIII proteins are the phage-specific proteins that are synthesized in the greatest amount both *in vivo* and *in vitro*. As shown in this paper, the bulk of the gene V protein appears to be synthesized by a number of different mRNAs. The most efficient, or at least the most abundant, of these are small RNA species that contain the information for only the gene V, VII, and VIII proteins. These RNA species direct the *in vitro* synthesis of more gene V protein than gene VIII protein, suggesting some type of translational polarity. This conclusion is supported by the observation that when these mRNAs

contain an amber mutation in the gene V region, they direct the synthesis of very little gene VIII protein. As discussed above, a number of these small gene V-containing mRNAs may occur as a result of processing from larger species. Therefore, the high amount of gene V protein synthesized in the cell could be due partially to processing reactions leading to a constantly replenished pool of small mRNAs that could be translated efficiently for the gene V protein.

While processing appears to result in the formation of various RNA species coding for the gene VIII protein, those that also code for the gene V protein may not be used efficiently for the production of this protein. Instead, it is probable that a large amount of gene VIII protein is synthesized by using the approximately 412-nucleotide RNA (band H). Our results and those of Rivera *et al.* (27) indicate that as much as 2% of the label incorporated into RNA is in this species. Consistent with the high level of synthesis is the suggestion that the promoter for this RNA is operating at a high level *in vivo* since it is not affected by an increase in temperature as the other promoters appear to be.

Further investigations on the nucleotide sequence of these mRNAs are necessary to identify the promoters and terminator used *in vivo*. In addition, sequence studies may be useful in clarifying the mechanism of the possible processing reactions.

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