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Transcription of Bacteriophage M13 DNA: Existence of Promoters Directly Preceding Genes III, VI, and I

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In vitro transcription and coupled transcription-translation studies have been performed with restriction fragments of bacteriophage M13 replicative-form DNA which contain either gene III, gene VI, or gene I. It could be demonstrated that DNA fragments which contain only gene III were able to direct the synthesis of gene III protein. Fragments which encompassed genes VI and I gave rise to the synthesis of gene I protein only, whereas gene I-containing fragments were able to direct the synthesis of gene I protein. None of the fragments studied gave rise to a detectable level of gene VI protein, although an RNA transcript of gene VI could readily be obtained during in vitro transcription of the relevant gene VI-containing DNA fragments. From these results we have concluded that the promoters $A_{0.44}$ and $A_{0.49}$ are located in front of genes VI and I, respectively, and that gene III is also equipped with a promoter ($X_{0.25}$). Introduction of a single cleavage within the gene III region does not abolish the expression of genes VI and I in vitro. Hence, the expression of these genes is not solely dependent on the initiation of RNA synthesis at the gene III promoter or on leakage of transcription through the central termination site ($T_{0.25}$), but is also determined by the initiation frequency of RNA synthesis at their individual promoters.

Control of transcription requires that initiation, as well as termination, occurs at appropriate sites on the DNA template. For a better understanding of this process, a detailed knowledge of the positions and respective efficiencies of these sites is essential.

During the last few years the in vitro transcription process of the filamentous phages has been studied extensively (for reviews, see references 2 and 16). From the results of these studies we now know that transcription of the M13 genome starts at eight promoter sites and that termination of transcription occurs at only a single site. Three of these promoters initiate the synthesis of RNA chains which start with pppA (A-promoters), whereas the other five promoters initiate RNA synthesis which starts with pppG (G-promoters) (5, 12, 18). It has also been demonstrated that the G-promoters are located immediately in front of genes VIII, V, X, and II (1, 4, 5, 21). Of the A-promoters, so far only one has been positioned on the genetic map of phage M13. By transcription and translation experiments it has been shown unambiguously that this A-promoter is located immediately in front of gene IV (8, 21). The genetic positions of the two remaining A-promoters are not known, although from transcription data their positions on the physical map have been calculated (5). Their calculated positions suggest a location immediately in front of genes VI and I. Direct proof for this assumption, however, is still lacking.

In the absence of rho factor, only one strong termination signal operates on the M13 genome (3, 4, 12). This central termination site has been localized in the intercistronic region between genes VIII and III (1, 3, 4, 20). Under in vitro conditions the termination of transcription at this site is not stringent. Approximately 10% of the RNA polymerase molecules leak through the central termination site and enhance the transcription frequency of the genes which are located distal to this site (4, 5; L. Edens, Ph.D. thesis, University of Nijmegen, Nijmegen, The Netherlands, 1978). Since up to now transcription studies have not given evidence for the existence of a strong promoter in front of gene III (4, 5, 12, 18), it has been postulated that the expression of gene III is accomplished by this read-through effect. However, the existence of a promoter in front of gene III with a very weak initiation capacity could not be excluded (1, 6, 12).

In this study we focused our attention on the question of whether genes III, VI, and I are preceded by promoter sites. Therefore, we isolated several restriction fragments which contained either the complete gene III, gene VI, or gene I and analyzed the template function of each fragment in both transcription and trans-

lation experiments. Upon translation of the DNA fragments in a DNA-dependent protein-synthesizing system, the synthesis of the proteins encoded by both gene III and gene I could be demonstrated. From these results we concluded that these genes are preceded by promoter sites. A similar conclusion was reached from the results of transcription studies of the relevant DNA fragments.

MATERIALS AND METHODS

Enzymes. *Escherichia coli* RNA polymerase holoenzyme was a generous gift from R. van Meteren (University of Leiden). The restriction endonuclease from *Arthrobacter luteus* (endo R·*Alu* I) was a kind gift from P. Baas (University of Utrecht). The restriction endonucleases from *Haemophilus aegyptius* (endo R·*Hae* II and endo R·*Hae* III) were isolated as described previously (22, 24). The restriction endonuclease endo R·*Bam*HI was obtained from Boehringer, Mannheim, Germany.

RF and restriction fragments. The method for the preparative isolation and purification of M13 replicative form I DNA (M13 RF) has been described (24). Digestion of M13 RF with the restriction endonucleases was performed as described by van den Hondel and Schoenmakers (23, 24). After digestion, the fragments were separated by electrophoresis on 2.5% (for endo R·*Hae* II and endo R·*Alu* I fragments) or 3.0% discontinuous polyacrylamide slab gels and further purified by sucrose density gradient centrifugation as described by van den Hondel et al. (25).

RNA synthesis in vitro. RNA synthesis in vitro was performed in a standard reaction mixture (0.1 ml) which contained: Tris-hydrochloride (pH 7.9), 4 μmol; KCl, 15 μmol; $MgCl_2$, 0.8 μmol; dithiothreitol, 0.1 μmol; EDTA, 0.01 μmol; UMP, 0.1 μmol; Tween 80, 0.1%; bovine serum albumin, 25 μg; M13 DNA restriction fragment, 0.2 pmol; and *E. coli* RNA polymerase holoenzyme, 8.0 pmol. After a preincubation period of 5 min at 37°C, ribonucleoside triphosphates were added to a final concentration of 80 μM, except for [α-^{32}P]-CTP, the concentration of which was 8 μM. After further incubation for 10 min, the reactions were terminated by the addition of 10 μl of 10% sodium dodecyl sulfate and 0.1 ml of phenol saturated with a buffer containing 0.01 M Tris-hydrochloride (pH 7.6) and 0.001 M EDTA.

After RNA synthesis, 10 μg of carrier tRNA was added, and the reaction mixtures were extracted with phenol. Subsequently, the RNA was precipitated twice with 2.5 volumes of cold ethanol and finally dissolved in 15 μl of deionized formamide. The RNA products synthesized in vitro were then analyzed on 1.9% polyacrylamide slab gels (20 by 20 by 0.15 cm) in the presence of 7 M urea (4).

Protein synthesis. Cell-free protein synthesis under the direction of restriction fragments was accomplished as described previously (8, 9). The polypeptides synthesized were analyzed on 15% sodium dodecyl sulfate–Tris–glycine polyacrylamide gels in the presence of 8 M urea (8).

Nomenclature of promoter sites. Promoters are denoted by A or G, depending on the ribonucleoside triphosphate with which RNA synthesis is initiated at the particular promoter site. The position of each promoter on the physical map is given in map units (1 map unit corresponds to a genome length of 6,400 bases), using the *Hin*dII cleavage site as a reference (zero) point. Promoters have been numbered corresponding to the direction of transcription.

RESULTS

Expression of gene III. In the absence of rho factor, only one strong termination site is operative on the M13 genome. This central termination site is located immediately distal to gene VIII but proximal to gene III (1, 3, 4, 20). Consequently, the expression of gene III may be accomplished either by a promoter in front of this gene or by a mechanism which allows leakage of RNA polymerase molecules through the termination site. Transcription studies have failed so far to detect a strong promoter in front of gene III, although the possibility that gene III is preceded by a promoter with a very low initiation capacity cannot be excluded. Therefore, we have studied that region of the genome which encompasses gene III in more detail.

To exclude the expression of gene III via a read-through mechanism, we isolated a template which codes for the complete gene III but contains none of the known G-promoter sites proximal to the central termination site. As shown in Fig. 1, the restriction fragment *Alu* I-A fulfills these requirements. From our transcription studies (3) and nucleotide sequence data presented elsewhere (Edens, Ph.D. thesis; T. Hulsebos and J. G. G. Schoenmakers, unpublished data), we know that the 5′ end of restriction fragment *Alu* I-A is located 46 nucleotides proximal to the central termination site. (The direction of transcription proceeds counterclockwise on the conventional genetic map. This is also the 5′ to 3′ polarity in the viral strand of each restriction fragment.) Consequently, this fragment does not contain any of the known G-promoters. The 3′ end of fragment *Alu* I-A is adjacent to restriction fragment *Alu* I-G, which contains genetic markers for both genes VI and I (22). Because the order of genes is III-VI-I and fragment *Alu* I-G is relatively small, it is most likely that the coding information for the N-terminal part of gene VI is located at the 3′ end of restriction fragment *Alu* I-A.

As shown in Fig. 2a, transcription of fragment *Alu* I-A gives rise to the synthesis of two discrete RNA species. The largest of these RNA products has a length of about 1,300 nucleotides (15S), whereas the smaller product is only about 200 nucleotides (6S) long. This observation already indicates that two RNA initiation sites are pres-

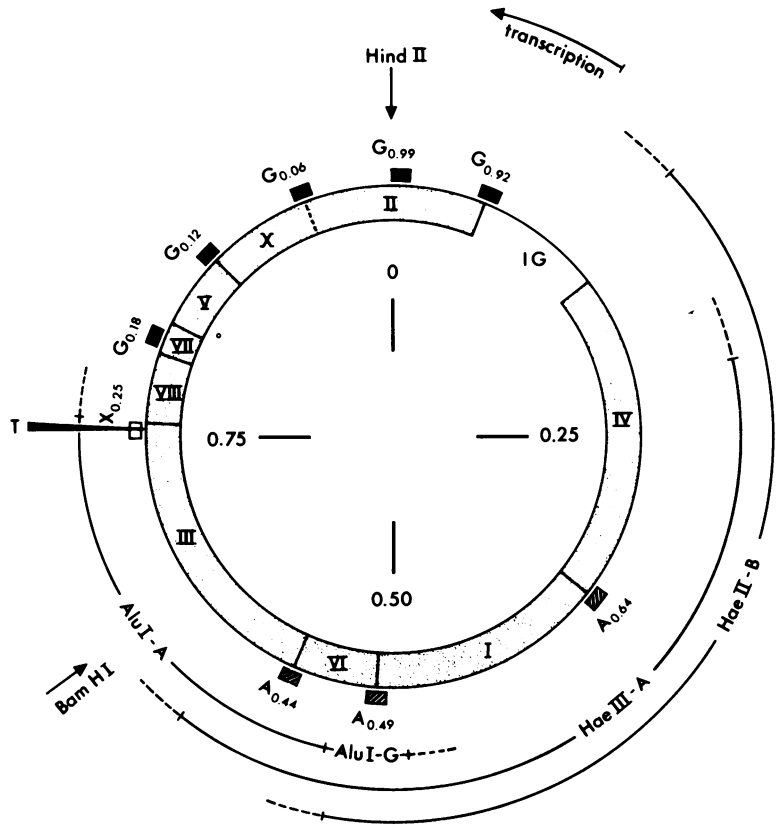

FIG. 1. *Genetic map and physical maps of bacteriophage M13 DNA. The dotted circular area represents the genetic map. The uncompleted outer circles show the locations of restriction fragments containing specific parts of the M13 genome. The positions of the G-promoters, A-promoters, and the gene III promoter are indicated with black bars, hatched bars, and an open bar, respectively. The latter promoter forms an integral part of the central termination site of transcription (T). The direction of transcription is counterclockwise around the genetic map. The arrows indicate the single cleavage sites of M13 RF by endo R·HindII and endo R·BamHI, respectively. IG refers to the intergenic region between genes II and IV in which the replication origin for complementary and viral strand DNA synthesis is located.*

ent on this fragment. Because fragment *Alu* I-A is 1,430 base pairs long (22) and transcription proceeds in a counterclockwise direction on the physical map (11, 21), one may conclude that one RNA initiation site must be located near the 5′ end of this fragment. In a similar way one might conclude that the second RNA initiation site is located at a distance of about 200 nucleotides from the 3′ end of the DNA fragment, i.e., at 0.43 map unit of the physical map. This observation is in complete agreement with our previous results from which we calculated that an A-promoter is located at 0.44 map unit (5). Therefore, it is probable that the small RNA product is initiated at this A-start promoter and is terminated at the 3′ end of fragment *Alu* I-A.

If the observed weak initiation of RNA synthesis at the 5′ end of fragment *Alu* I-A is due to a promoter which is located in front of gene III, fragment *Alu* I-A must be able to direct the synthesis of gene III protein in a DNA-dependent protein-synthesizing system. To test this hypothesis, M13 RF was cleaved with an excess of restriction enzyme endo R·*Alu* I, and the complete digest was directly translated in a coupled transcription-translation system. From the results which are shown in Fig. 3d, it is clear that the endo R·*Alu* I-cleaved RF is still able to direct the synthesis of a protein with a molecular weight identical to the molecular weight of gene III protein (Fig. 3g). Since such a protein is not made when translation is carried out under the direction of a complete endo R·*Alu* I digest of M13 RF carrying various *am*3 mutations (Fig. 3a to c), it is almost certain that the high-molecular-weight protein is encoded by gene III. Translation of the isolated restriction fragment *Alu* I-A (Fig. 3e) confirms our presumption that

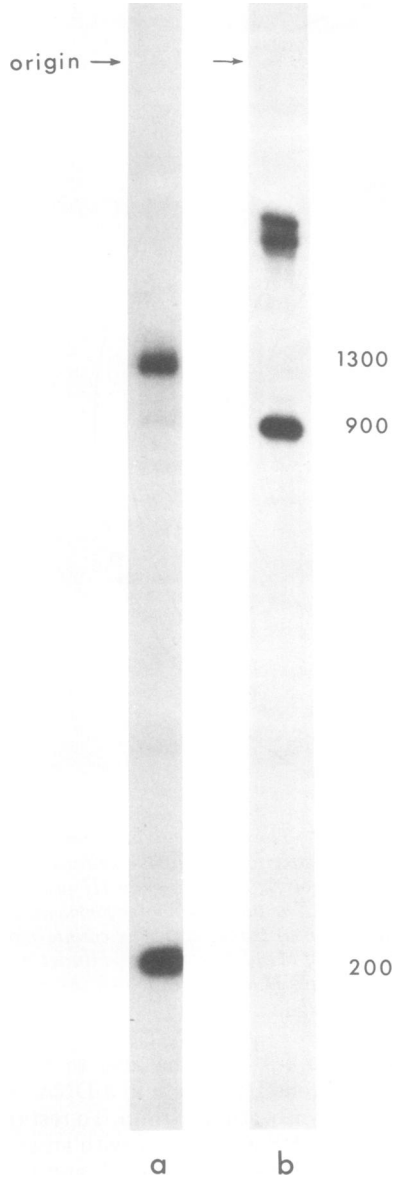

FIG. 2. *Autoradiogram of ^{32}P-labeled RNA species synthesized in vitro under the direction of restriction fragments of M13 RF. (a) Products transcribed from fragment Alu I-A; (b) products transcribed from fragment Hae III-A. RNA synthesis was carried out in the presence of [α-^{32}P]CTP and was performed as described in the text. The RNA products were analyzed on vertical 1.9% acrylamide slab gels in the presence of 7 M urea. The nucleotide lengths of the RNA products indicated were estimated as described previously (5).*

this particular DNA fragment is responsible for the observed synthesis of gene III protein. Thus, both transcription and translation studies strongly indicate the existence of a (weak) promoter in front of gene III.

Expression of gene I. Three of the promoters which are located on the M13 genome initiate RNA synthesis which starts with pppA. Although the map positions of all of these A-promoters are known exactly, up to now only the strong promoter $A_{0.64}$ has been localized on the genetic map of the M13 genome. Its position was found immediately in front of gene IV (8, 21).

To also determine the positions of the remaining A-promoters, we studied the transcription properties of restriction fragment *Hae* III-A. This fragment encompasses the C-terminal part of gene III, the entire gene VI and gene I, and about 1,000 base pairs of the N-terminal part of gene IV (21, 25). Upon transcription of this DNA fragment, three RNA products are formed, the sizes of which are about 900, 1,700, and 2,100 nucleotides, respectively (Fig. 2b). Given the assumption that the termination of transcription occurs at the 5′ end of the codogenic strand of each fragment, the estimated lengths of these RNA species confirm a starting point of RNA chain growth at the previously identified promoters $A_{0.64}$, $A_{0.49}$, and $A_{0.44}$ (5). Since the latter two promoters are located on the genetic map in a region where several amber mutations of genes VI and I have also been mapped (22, 25), it is tempting to speculate that these two A-promoters are located immediately in front of genes VI and I. If this is true, restriction fragment *Hae* III-A must be able to direct the synthesis of both proteins. Translation of fragment *Hae* III-A in a coupled transcription-translation system clearly shows the presence of a protein which comigrates with gene I protein synthesized under the direction of intact M13 RF (Fig. 4b and f). The synthesis of this protein, i.e., gene I protein, also occurs with M13 RF that has been cleaved once with endo R·*Hin*dII (Fig. 4d). Synthesis of gene I protein, however, is not directed by M13 RF derived from M13 phage DNA carrying an amber mutation in gene I (Fig. 4c). We are confident, therefore, that fragment *Hae* III-A is capable of directing the synthesis of gene I protein and that at least one of the two A-promoters must be located in front of gene I. The presence of gene VI protein among the translational products, however, could not be demonstrated. Its synthesis is apparent neither on fragment *Hae* III-A nor on intact M13 RF. In fact this is not surprising since the synthesis of this protein has not been detected either in vitro (8, 11) or in vivo (7, 19). Due to this failure, a conclusion as to whether gene VI is also equipped with a promoter cannot be drawn.

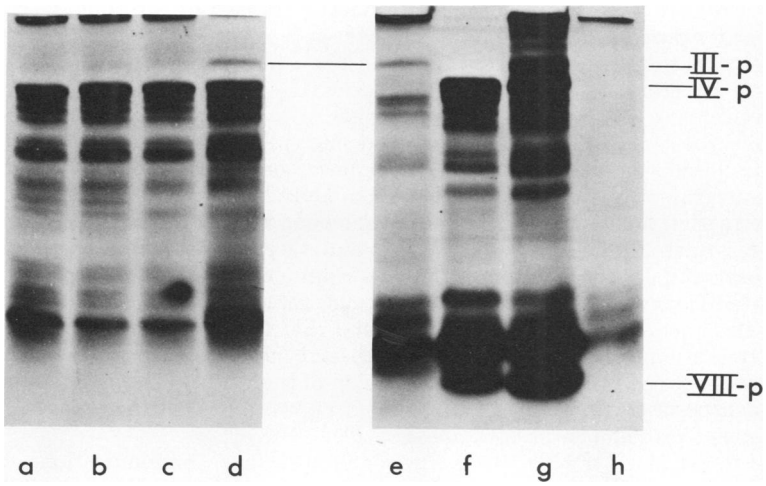

FIG. 3. *Autoradiogram of [^{35}S]methionine-labeled polypeptides synthesized in vitro under the direction of either wild-type M13 RF, amber mutant RF, or complete endo R·Alu I digests of M13 RF. Polypeptides synthesized under the direction of the complete endo R·Alu I digests of M13 RF bearing (a) the am3-H5 mutation, (b) the am3-H4 mutation, and (c) the am3-H1 mutation; (d) complete endo R·Alu I digest of wild-type M13 RF; (e) purified fragment Alu I-A; (f) intact RF bearing the am3-H5 mutation; (g) intact wild-type RF; (h) products synthesized in the absence of exogenous DNA. Protein synthesis in vitro was carried out as described previously (8, 9). An amount equivalent to 1 μg of RF of each template was added per 25 μl of reaction mixture. The polypeptides synthesized in vitro were analyzed on 15% sodium dodecyl sulfate-Tris-glycine gels (8). III-P, IV-P, and VIII-P refer, respectively, to the positions of migration of the products of gene III, gene IV, and gene VIII. Mutants am3-H1, am3-H4, and am3-H5 have their amber mutation in different positions within gene III (22).*

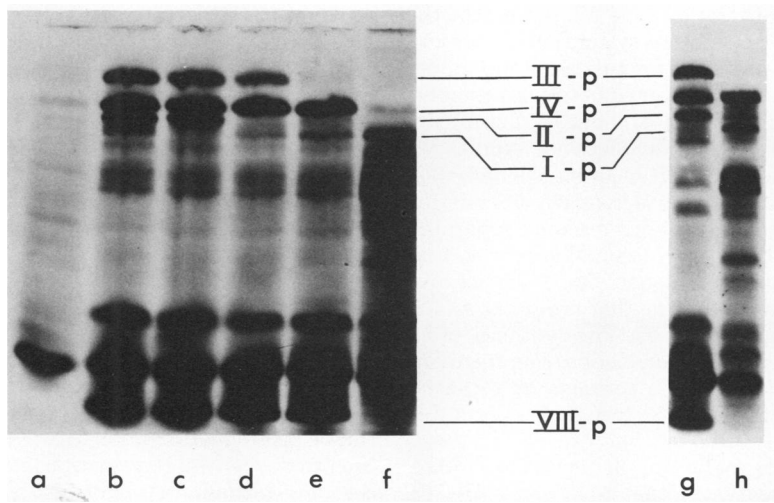

FIG. 4. *Autoradiogram of [^{35}S]methionine-labeled polypeptides synthesized in vitro under the direction of either wild-type M13 RF, amber mutant RF, or various restriction fragments. (a) Products synthesized in the absence of exogenous DNA; (b) products synthesized under the direction of intact wild-type M13 RF I; (c) M13 RF I bearing the am1-H6 mutation; (d) M-13 RF cleaved with endo R·HindII; (e) M13 RF cleaved with endo R·BamHI; (f) purified fragment Hae III-A; (g) M13 RF bearing the am1-H7 mutation; (h) purified fragment Hae II-B. Protein synthesis was carried out as described previously (8). Further conditions are described in the legend to Fig. 3. I-P, II-P, etc., refer, respectively, to the positions of migration of the products of gene I, gene II, etc.*

To establish whether gene I is preceded by promoter $A_{0.44}$ or by promoter $A_{0.49}$, translation experiments were performed with restriction fragment *Hae* II-B. From its position on the genetic and physical map (22), we know that this fragment contains the complete gene I and gene IV (Fig. 1). Nucleotide sequence analyses have shown that this fragment contains in addition, the coding information for 49 amino acid residues of the C-terminal end of gene VI protein (P. van Wezenbeek and J. G. G. Schoenmakers, unpublished data). Hence, promoter $A_{0.44}$ is not encompassed in fragment *Hae* II-B. As shown in Fig. 4h, upon translation of fragment *Hae* II-B, not only is the complete gene IV protein synthesized, but the synthesis of a protein which comigrates with gene I protein synthesized under the direction of intact M13 RF (Fig. 4b) is also apparent. The latter protein is absent upon translation of M13 RF carrying an amber mutation in gene I (fig. 4g). From these results we conclude that the capacity of directing the synthesis of gene I protein is still preserved on fragment *Hae* II-B, and, consequently, promoter $A_{0.49}$ is the one which must be located immediately in front of gene I.

Because promoter $A_{0.44}$ is positioned upstream of promoter $A_{0.49}$ and gene VI is located proximal to gene I (Fig. 1), it is attractive to postulate that $A_{0.44}$ is the promoter which is positioned in front of gene VI. However, due to the absence of detectable synthesis of gene VI protein in the DNA-dependent cell-free system, no direct evidence can be provided for this assumption. On the other hand, the distance between promoter $A_{0.44}$ and promoter $A_{0.49}$ is about 350 base pairs (5). From our nucleotide sequence studies we now know that gene VI encompasses only 339 base pairs and that the regions which code for gene VI and gene I proteins are only separated by a single base pair (van Wezenbeek and Schoenmakers, unpublished data). Hence, we feel justified in concluding that promoter $A_{0.44}$ is located in front of gene VI. The synthesis of an RNA species of only 200 nucleotides on fragment *Alu* I-A (Fig. 2a), which contains only the N-terminal part of gene VI, strongly supports this conclusion.

Based upon genetic complementation studies, it has been suggested that M13 genes III, VI, and I form a regulatory unit which can only be expressed via a single polycistronic mRNA (10, 14). To determine whether this is true, M13 RF which had been cleaved with endo R·*Bam*HI was used. The latter enzyme has only a single recognition site on the M13 RF molecule and cleaves the DNA genome within gene III (Fig. 1; van Wezenbeek and Schoenmakers, unpublished data). Translation of endo R·*Bam*HI-cleaved RF in a DNA-dependent protein-synthesizing system shows, as expected, that the synthesis of gene III protein is absent (Fig. 4e). If the expression of genes III, VI, and I occurs on polycistronic mRNA's, as suggested by the polarity effects, synthesis of gene I protein should also be abolished on this template. However, as shown in Fig. 4e, this is not the case. From these results we conclude that the initiation of transcription of genes VI and I at the promoter sites immediately preceding these genes is retained and that transcription of gene III is not, under these conditions, a prerequisite for the expression of the distal genes.

DISCUSSION

In previous communications we have used transcription and translation studies of restriction fragments to demonstrate the existence of promoters in front of particular genes. The capability of a DNA fragment or of its transcript to direct the in vitro synthesis of a phage-specific polypeptide was used as an indication of whether a promoter is located in front of the gene coding for this polypeptide. Using this approach, we could demonstrate that promoters $G_{0.18}$, $G_{0.12}$, $G_{0.92}$, and $A_{0.64}$ are, respectively, located immediately in front of genes VIII, V, II, and IV. In addition, it was found that promoters $G_{0.06}$ and $G_{0.99}$ are intragenic RNA initiation sites which are located within gene II. For promoter $G_{0.06}$ it furthermore was found that this is located in front of a gene, i.e., gene X, whose nucleotide sequence completely overlaps with the C-terminal end of gene II.

By the same strategy we have now presented evidence that promoters $A_{0.44}$ and $A_{0.49}$ belong to genes VI and I and that there also exists a promoter, i.e., $X_{0.25}$, immediately in front of gene III.

Although our protein synthesis data clearly demonstrated the existence of a gene III promoter, the inaccuracy of length measurements of RNA chains on polyacrylamide gels did not allow a more precise location of this promoter to be made. Theoretically, the gene III promoter can be located either immediately distal or proximal to the central termination site. In the latter case the terminator might also function as an attenuator which regulates the expression of gene III. A proximal position, however, is very unlikely since in that case the gene III promoter should generate very short RNA chains which are prematurely terminated at the termination site. Upon transcription of the appropriate restriction fragment, such short RNA chains with a characteristic U_8 track at their 3'-OH terminus

(see reference 20) have never been detected among the transcriptional products (Edens, unpublished data). Recent nucleotide sequence analyses of Takanami and co-workers (personal communication) have now revealed that the gene III promoter of phage fd forms an integral part of the central termination site. Since the nucleotide sequence of the terminator region on the M13 genome has been found to be completely identical to the one deduced for phage fd (20; Edens, Ph.D. thesis), it is almost certain that the observed gene III promoter on the M13 genome is also located within the central terminator region. Such a position also explains the observed diminished synthesis of gene III protein on endo R·*Alu* I-treated RF as compared with intact superhelical RF (Fig. 3). The endo R·*Alu* I cut is 46 nucleotides proximal to the central terminator and, hence, might influence the gene III promoter function. From our nucleotide sequence analyses we learned that the primary "binding sequence" (see references 15 and 17) of the gene III promoter is still preserved in fragment *Alu* I-A, but that the second, so-called "recognition site" (15), although not being an absolute necessity for proper RNA polymerase binding and RNA chain initiation (13), is missing. The diminished gene III protein synthesis is, therefore, probably caused by a partly destroyed promoter function.

Additional effects on gene III protein synthesis cannot be ruled out, however. We previously demonstrated that the termination of transcription at the central termination site—at least under the in vitro conditions applied—is not stringent (4, 5). Since the endo R·*Alu* I cut is immediately proximal to the central terminator, this will additionally exert a reduction of gene III protein synthesis due to a switch off of read through of transcription at this particular site. To what extent this read through contributes to the expression of gene III cannot be answered yet since proper (linear) RF templates cannot yet be constructed on which read through is completely eliminated without a concomitant disturbance of gene III promoter function. A more general problem underlying such in vitro transcription-translation studies is that RNA initiation frequencies will differ on DNA templates with different configurations. It has already been demonstrated by Seeburg et al. (17) that the RNA polymerase binding capacity is diminished at least 10-fold from supercoiled to relaxed RF. In our opinion, this reduction in complex formation and, hence, in RNA initiation is not an overall effect but might be completely different for each individual promoter. This is clearly recognized by comparing the patterns obtained with intact RF (Fig. 4b) with those obtained from endo R·*Hin*dII-treated RF (Fig. 4d) and endo R·*Bam*HI-treated RF (Fig. 4e). The amount of gene II protein is high on supercoiled RF and is absent, as expected, on RF in which an endo R·*Hin*dII cut is introduced into gene II. Introduction of an enzyme cut into a completely different region, i.e., within gene III, also diminishes the synthesizing capacity of gene II protein to a very large extent. Therefore, a direct comparison of the initiation frequency for a given promoter on various linear and supercoiled RF molecules cannot be made and limits the interpretations of results in which these different DNA templates have been applied.

Another aspect of our results concerns the polarity observed in vivo among genes III, VI, and I. The operon model, which has been postulated to be pertinent for these genes (10, 14), implies that the introduction of a cleavage within gene III also prevents the expression of distal genes VI and I. Our in vitro results from the coupled transcription and translation system, however, suggest the opposite since an interruption of gene III transcription did not abolish the synthesis of gene I protein. This result is in accordance with the existence of promoters in front of genes VI and I. If these promoters also function as such in infected cells, the operon model is no longer applicable for these genes, and other regulatory mechanisms have to be reconsidered. Further studies, such as in vitro rearrangements of genes and a detailed analysis of the in vivo RNA species, are needed to clarify these contradictory results on polarity.

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LITERATURE CITED

1. **Chan, T., P. Model, and N. D. Zinder.** 1975. *In vitro* protein synthesis directed by separated transcripts of bacteriophage f1 DNA. J. Mol. Biol. **99**:369–382.
2. **Denhardt, D. T.** 1975. The single-stranded DNA phages. CRC Crit. Rev. Microbiol. **4**:116–123.
3. **Edens, L., R. N.H. Konings, and J. G. G. Schoenmakers.** 1975. Physical mapping of the central terminator for transcription on the bacteriophage M13 genome. Nucleic Acid Res. **2**:1811–1820.
4. **Edens, L., R. N. H. Konings, and J. G. G. Schoenmakers.** 1978. A cascade mechanism of transcription in bacteriophage M13 DNA. Virology **86**:354–367.
5. **Edens, L., P. van Wezenbeek, R. N. H. Konings, and J. G. G. Schoenmakers.** 1976. Mapping of promoter sites on the genome of bacteriophage M13. Eur. J. Biochem. **70**:577–587.
6. **Giacomani, P. U., E. Delain, and J. B. le Pecq.** 1977. Electron microscopy analysis of the interaction between *Escherichia coli* DNA-dependent RNA polymerase and the replicative form of phage fd DNA. I. Mapping

of the binding sites. Eur. J. Biochem. **78**:205–213.
7. **Henry, T. J., and D. Pratt.** 1969. The proteins of bacteriophage M13. Proc. Natl. Acad. Sci. U.S.A. **62**:800–807.
8. **Konings, R. N. H., T. Hulsebos, and C. A. van den Hondel.** 1975. Identification and characterization of the in vitro synthesized products of bacteriophage M13. J. Virol. **15**:570–584.
9. **Konings, R. N. H., R. Ward, B. Francke, and P. H. Hofschneider.** 1970. Gene order of RNA bacteriophage M12. Nature (London) **266**:604–607.
10. **Lyons, L. B., and N. D. Zinder.** 1972. The genetic map of the filamentous bacteriophage f1. Virology **49**:45–60.
11. **Model, P., and N. D. Zinder.** 1974. *In vitro*-synthesis of bacteriophage f1 proteins. J. Mol. Biol. **83**:231–251.
12. **Okamoto, T., K. Sugimoto, H. Sugisaki, and M. Takanami.** 1975. Studies on bacteriophage fd DNA II. Localisation of RNA-initiation sites on the cleavage map of the fd genome. J. Mol. Biol. **95**:33–44.
13. **Okamoto, T., K. Sugimoto, H. Sugisaki, and M. Takanami.** 1977. DNA regions essential for the function of a bacteriophage fd promoter. Nucleic Acid Res. **4**:2213–2222.
14. **Pratt, D., H. Tzagoloff, W. S. Erdahl, and T. J. Henry.** 1967. Conditional lethal mutants of coliphage M13, p. 219. *In* J. S. Colter and W. Parachych (ed.), Molecular biology of viruses. Academic Press Inc., New York.
15. **Pribnow, D.** 1975. Bacteriophage T7 early promoters: nucleotide sequences of two RNA polymerase binding sites. J. Mol. Biol. **99**:419–443.
16. **Ray, D. S.** 1977. Replication of filamentous bacteriophages, p. 105–178. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology. Plenum Publishing Corp., New York.
17. **Seeburg, P. H., C. Nüsslein, and H. Schaller.** 1977. Interaction of RNA polymerase with promoters from bacteriophage fd. Eur. J. Biochem. **74**:107–113.
18. **Seeburg, P. H., and H. Schaller.** 1975. Mapping and characterization of promoters in phage fd, f1 and M13. J. Mol. Biol. **92**:261–277.
19. **Smits, M. A., G. Simons, R. N. H. Konings, and J. G. G. Schoenmakers.** 1978. Expression of bacteriophage M13 DNA *in vivo*. I. Synthesis of phage-specific RNA and proteins in minicells. Biochim. Biophys. Acta, in press.
20. **Sugimoto, K., H. Sugisaki, T. Okamoto, and M. Takanami.** 1977. Studies on bacteriophage fd DNA. IV. The sequence of messenger RNA for the major coat protein gene. J. Mol. Biol. **110**:487–507.
21. **van den Hondel, C. A., R. N. H. Konings, and J. G. G. Schoenmakers.** 1975. Regulation of gene activity in bacteriophage M13 DNA: coupled transcription and translation of purified genes and gene fragments. Virology **67**:487–497.
22. **van den Hondel, C. A., L. Pennings, and J. G. G. Schoenmakers.** 1976. Restriction enzyme cleavage maps of bacteriophage M13: existence of an intergenic region. Eur. J. Biochem. **68**:55–70.
23. **van den Hondel, C. A., and J. G. G. Schoenmakers.** 1975. Studies on bacteriophage M13 DNA. I. A cleavage map of the M13 genome. Eur. J. Biochem. **53**:547–558.
24. **van den Hondel, C. A., and J. G. G. Schoenmakers.** 1976. Cleavage maps of the filamentous bateriophages M13, fd, f1, and ZJ/2. J. Virol. **18**:1024–1039.
25. **van den Hondel, C. A., A. Wyers, R. N. H. Konings, and J. G. G. Schoenmakers.** 1975. Studies on bacteriophage M13 DNA. II. The gene order of the M13 genome. Eur. J. Biochem. **53**:559–567.