INSERTION OF TRANSPOSON TN5 INTO SPECIALIZED LAMBDA METHIONINE TRANSDUCING BACTERIOPHAGE

by

Mark L. Weaver

Department of Biology, Texas A&M University

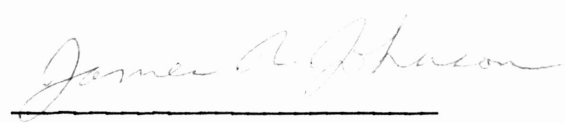
Submitted in Partial Fulfillment of

the Requirements of the University

Undergraduate Fellows Program

1979-1980

Approved by:



Dr. James R. Johnson

April, 1980

INSERTION OF TRANSPOSON TN5 INTO SPECIALIZED LAMBDA METHIONINE TRANSDUCING BACTERIOPHAGE

Mark Weaver

Department of Biology, Texas A&M University, College Station, TX 77843

SUMMARY. The transposon, Tn5, has successfully been inserted into specialized lambda transducing bacteriophage. These phage carry the *met* B, J, LM, and F methionine biosynthetic genes from *Escherichia coli*. The nature of this insertion (near the *met* genes) makes possible further characterization of the organization and regulation of these genes.

INTRODUCTION

Methionine is one of the twenty amino acid building blocks of all proteins, and it is the initiator of ribosomal mediated peptide synthesis. It is also involved in other important cellular biochemical processes (reviewed in Lehninger, 1975). Since human tissues can complete only the last step of methionine's complex biosynthetic pathway and cannot manufacture it *de novo* from simple carbohydrates, methionine is classified as an essential amino acid. That is, it must be supplied in the human diet. Inability to obtain sufficient amounts of this nutrient is a serious world health problem. The protein found in most crop plant

---

The format of this paper is taken from *Molecular and General Genetics*.

tissues is methionine deficient, as is the protein derived from alternative microbial single-cell methods (reviewed in Reisman, 1977 and Kharatzan, 1978). Consequently, methionine must be added to supplement many food protein sources. Industry makes this supplemental methionine by direct chemical synthesis from petroleum, an expensive starting material (Reisman, 1977).

The bacterium, Escherichia coli, can produce methionine de novo from elementary organic molecules (reviewed in Flavin, 1975). The genes responsible for this process are scattered on the E. coli chromosome (see Fig. 1). Methionine and S-adenosylmethionine regulate the pathway by feedback inhibition of the enzyme that converts homoserine into o-succinylhomoserine (the first committed step towards methionine). A second, not well understood regulatory mechanism is repression of met gene transcription by a presumed repressor protein (the product of the met J gene) and a corepressor (S-adenosylmethionine or a related derivative). Three of the biosynthetic genes, met B, LM, and F, as well as the primary regulatory gene, met J, are located in a cluster near the 87 minute point of the E. coli chromosome (Fig. 1). These genes have been cloned into the DNA of lambda bacteriophage (Johnson et al., 1977). Since lambda is a temperate phage, it can be a means of moving the DNA contained within it from one E. coli genetic background to another (reviewed in Luria et al., 1978).

To assist in the investigation of this genetic system I have employed a recently discovered genetic phenomenon, the transposable element, or transposon. A transposon is a discrete piece of DNA that carries drug resistance marker genes; it can insert within and excise from procaryotic DNA independently of host recombination mechanisms (Kleckner,

1977). I used the specific transposon, Tn5, which carries the genes for resistance to the antibiotics kanamycin and neomycin and contains recognized cleavage sites for many different DNA restriction endonucleases (Jorgensen et al., 1979).

This paper reports on original research aimed at using this transposon to adapt methionine transducing phage for molecular genetic study and manipulation. The first step is to insert Tn5 into the chromosomes of these lambda bacteriophage.

MATERIALS AND METHODS

Strains. E. coli K-12 strain JJ125 is a met BJLMF minus multiple mutant (J. Johnson, personal communication). Lambda dmet128 is a defective transducing phage carrying the met BJLMF gene cluster from E. coli in place of several stuctural genes normally found on the left arm of the lambda chromosome (Johnson et al., 1977). Figure 2 is a representation of the DNA of lambda and lambda dmet128. All lambda strains, including the wild type helper phage required to grow the dmet128 strain, contain the CI857 (temperature sensative repressor) allele. Tn5 was obtained from lambda bbkan2, a phage carrying two Tn5 elements (R. Young, personal communication).

Growth methods and media. E. coli cells were grown on LB medium or Davis-Mingioli minimal medium. Lysogens were grown on LB-lambda medium, except for large scale lysate cultures which were grown in tryptone broth. All of these media are as described in Johnson et al., 1977. Kanamycin selective media were prepared by adding kanamycin sulfate at a concentration

of 40 micrograms/ml. Static suspensions of cells were in deionized water with 0.9% NaCl and 0.01M $MgSO_4$. Static suspensions of viruses were in Zubay buffer (Johnson et al., 1977).

Purification methods. Viruses were purified by centrifugation in Cesium Chloride density gradients. Where appropriate, gradients were fractionated with an ISCO model 640 Fractionator monitored by an ISCO model UA5 Absorbance Monitor at 254 nm wavelength of light. Lysogens were cured of prophage by the heat pulse method: a fresh culture aliquot is diluted 1:100 into medium at 42°C, incubated 5 min., diluted 1:100 again into 0°C medium, incubated 30 min., and then restored to 32°C to grow overnight. Colonies obtained by plating this overnight culture on selective medium at 42°C were screened by the lambda superinfection immunity test (Miller, 1972) to make certain that the viral prophage had been lost.

Restriction enzyme analysis. Analysis of phage DNA was carried out as follows: DNA concentrations were adjusted to 20-50 micrograms/ml in 0.01 M TRIS, pH 7.5. To four parts DNA was added one part digestion buffer consisting of 0.5M TRIS, 0.25M NaCl, 0.025M $MgCl_2$ at pH 7.6. Samples were heated to 65°C for 3 min., then quenched in ice water. Two successive one hour digestions with endonucleases (5 microliters EcoRI or 0.5 microliters HindIII per 0.5 ml sample) were immediately performed at 37°C before stopping with an equal volume of stop solution consisting of 0.04M EDTA, 20% glycerol, 2% SDS, 0.02% bromophenol blue dye, and 4 milligrams/ml sea plaque agarose. Samples were again heated to 65°C for 3 min., and 5 microliter aliquots were subjected to horizontal agarose gel electrophoresis (0.4% agarose gel) at constant voltage of 15VDC. The gel and

electrode buffer contained 0.04M TRIS, 0.03M NaH_2PO_4, 0.001M EDTA, and one microgram/ml ethidium bromide at pH 7.5.

Photography of gels. Gels were illuminated from below with a UV light source emitting at a wavelength of 254 nm. Gels were photographed through a Kodak 23A orange gelatin filter and a Soligor UV filter using Ektapan film in a Polaroid MP3 Land camera.

RESULTS

Procedure for constuction of lambda dmet128::Tn5 bacteriophage. E. coli K-12 strain JJ125 was infected with lambda bbkan2, and kanamycin resistant lysogens were selected. Such cells were cured of the prophage (see methods) and kanamycin resistant non-lysogens were selected. Since the E. coli chromosome is 80 times larger than that of lambda, and Tn5 reinserts at a frequency of 10^{-3} per transposon per cell generation (Kleckner, 1977), it was not difficult to isolate JJ125::Tn5 cells.

Next, these transposon bearing cells were infected with lambda dmet128, a specialized transducing phage carrying the met BJLMF gene cluster. Since JJ125 lacks these genes and cannot make methionine, lysogens were easily selected by their ability to grow on minimal (methionine free) medium. Lysates of such cells were prepared and viruses carrying the transposon were selected by their ability to transduce both kanamycin resistance and methionine prototrophy to recipient JJ125 cells. It is important to realize that only the transducing phage can accept the transposon. The wild type helper phage cannot pick up the Tn5 element because its DNA is too large to be packaged into capsids along with Tn5. The

lambda dmet128 chromosome is about six kilobases shorter than wild type, and so it can easily acquire the Tn5 element (Fig. 2). Tn5 is 5.6 kilobases long (Jorgensen et al., 1979). 332 strains of lambda dmet128::Tn5 were obtained by this method.

Screening of Tn5 bearing viral strains. Lysogens of JJ125 and each of the 332 lambda dmet128::Tn5 strains were tested for a specific mutagenic site of transposon insertion. As Fig. 3 indicates, the met B gene product performs the step leading to production of cystathionine. Thus, met B minus mutants are unable to grow on minimal medium (since they lack one of the pathway enzymes) but do grow if supplemented with cystathionine. Replica plating of these lysogens on minimal and minimal plus cystathionine media (5mM cystathionine) revealed that all of them have functional met B genes. Since the only source of this gene for the lysogens is the lambda phage, none of these phage have Tn5 inserted in the met B gene.

This is a significant observation. Given that one gene is about 1000 base pairs long (Watson, 1970), then lambda dmet128 contains about 41 genes (J. Johnson, personal communication, lambda dmet128 is 41.5 kilobases long). One would predict, if Tn5 is random in its insertion, to find a met B insertion in one of every 41 separately isolated transposon carrying strains. I have found that this frequency is less than one in 332. Although this is preliminary data, it supports the findings of Shaw and Berg (1979) which suggest that Tn5 is, in fact, not random in its target site preferences. This contradicts earlier theories (Kleckner, 1977).

Upon abandoning the search for specific met gene insertions

(screening of 50 strains for met F insertions was similarly unsuccessful) the question arose: where is the transposon inserted in these viruses? Characterization of strain 77 (lambda dmet128::Tn5 MW77) has yielded the following information:

Since the insertion of Tn5 (5.6 kilobases) into lambda dmet128 (41.5 kilobases) brings the new DNA's size virtually up to that of the helper phage (48 kilobases), the transposon carrying strain 77 cannot be purified from the helper virus even by high speed density gradient ultracentrifugation (see methods). However, fractionation of such a preparation (Fig. 4) shows a slight separation of strain 77 particles and helper phage virions due to the approximately 900 base pair difference in their genomes. DNA molecules extracted from this fractionation were digested with EcoRI endonuclease and analyzed by electrophoresis (see methods). Figure 5 is a photograph of such a gel.

A standard curve of log molecular weight verses relative migration distances of EcoRI restriction fragments (Fig. 6) was prepared from the lambda wild type control of this gel using molecular weight information contained in Daniels et al. (1980). From this data one may calculate that the shift of the topmost lambda dmet128 band to a higher position in the 77 strain fractions represents a difference of approximately six kilobases worth of DNA. This is what one would predict from an insertion of the Tn5 element into this DNA fragment. This EcoRI fragment is the one containing the met gene cluster on the lambda dmet128 chromosome (Fig. 2).

A further digestion of these DNA molecules with both EcoRI and HindIII enzymes (data not shown) reveals that the Tn5 element (which carries two HindIII cleavage sites) is located about 1000 base pairs

from one end or the other of the EcoRI fragment containing the met bacterial genes. It is probably inserted one kilobase from the left end of this fragment (Fig. 7) since an insertion one kilobase from the right end would place it within the met F gene. The met F gene of strain 77 is known to be functional. The transposon can be located with certainty only by heteroduplex analysis, but this must await purification of lambda dmet128::Tn5 MW77 (which is, as mentioned previously, accompanied by some technical problems). Efforts to utilize a more suitable helper phage are in progress.

DISCUSSION

Insertion of the transposable element, Tn5, has been acheived in lambda methionine transducing bacteriophage. When such an insertion is not in a recognizable gene (as appears to be the case in strain 77) it is much easier to locate the transposon in the small viral genetic background than in the large bacterial system. Even though the insertion of Tn5 is not random and is not prefered in met B or met F, it may still be possible to obtain transposon mediated mutagenesis of met genes in this viral system as have been obtained (in small numbers) in the bacterial system (Shaw and Berg, 1979). Regardless of specific mutations, the endonuclease cleavage sites of Tn5 make possible new genetic manipulations of the DNA into which it has inserted. And, of course, the drug resistance characteristic provides a simple and sraightforeward selection device for transposon bearing viruses.

Acknowledgements. The author wishes to thank Dr. James Johnson of the

Department of Biology, Texas A&M University for supplying equipment, assistance, and his extensive and valuble expertise in support of this research.

REFERENCES

Daniels, D., deWet, J., and Blattner, F.: New map of bacteriophage lambda DNA. J. of Virol. 33, 390-400 (1980)

Flavin, M.: Methionine biosynthesis. IN Greene, R.: Metabolism of Sulfur Compounds, Vol. VII, Metabolic Pathways. Academic Press (1975)

Johnson, J., Greene, R., and Krueger, J.: Isolation and characterization of specialized lambda transducing bacteriophage carrying the met BJF gene cluster. J. of Bact. 131, 795-800 (1977)

Jorgensen, R., Rothstein, S., and Reznikoff, W.: A restriction enzyme cleavage map of Tn5 and location of a region encoding neomycin resistance. Molec. gen. Genet. 177, 65-72 (1979)

Kharatzan, S.: Microbes as human food. Ann. Rev. Of Microbiol. 32, 301-327 (1978)

Kleckner, N.: Translocatable elements in procaryotes (Review). Cell. 11, 11-23 (1977)

Lehninger, A.: Biochemistry. Worth Publishers (1975)

Luria, S., Darnell, J., Baltimore, D., and Campbell, A.: General Virology. Wiley and Sons, Inc. (1978)

Miller, J.: Experiments in Molecular Genetics. Cold Spring Harbor (1972)

Reisman, H.: Amino acids. IN McKetta, J. and Cunningham, W., eds.: Encyclopedia of Chemical Processing and Design. Decker Press (1977)

Shaw, K., and Berg, C.: Escherichia coli K-12 auxotrophs induced by insertion of the transposable element Tn5. Genetics. 92, 741-747 (1979)

Watson, J.: Molecular Biology of the Gene. W.A. Benjamin, Inc. (1970)

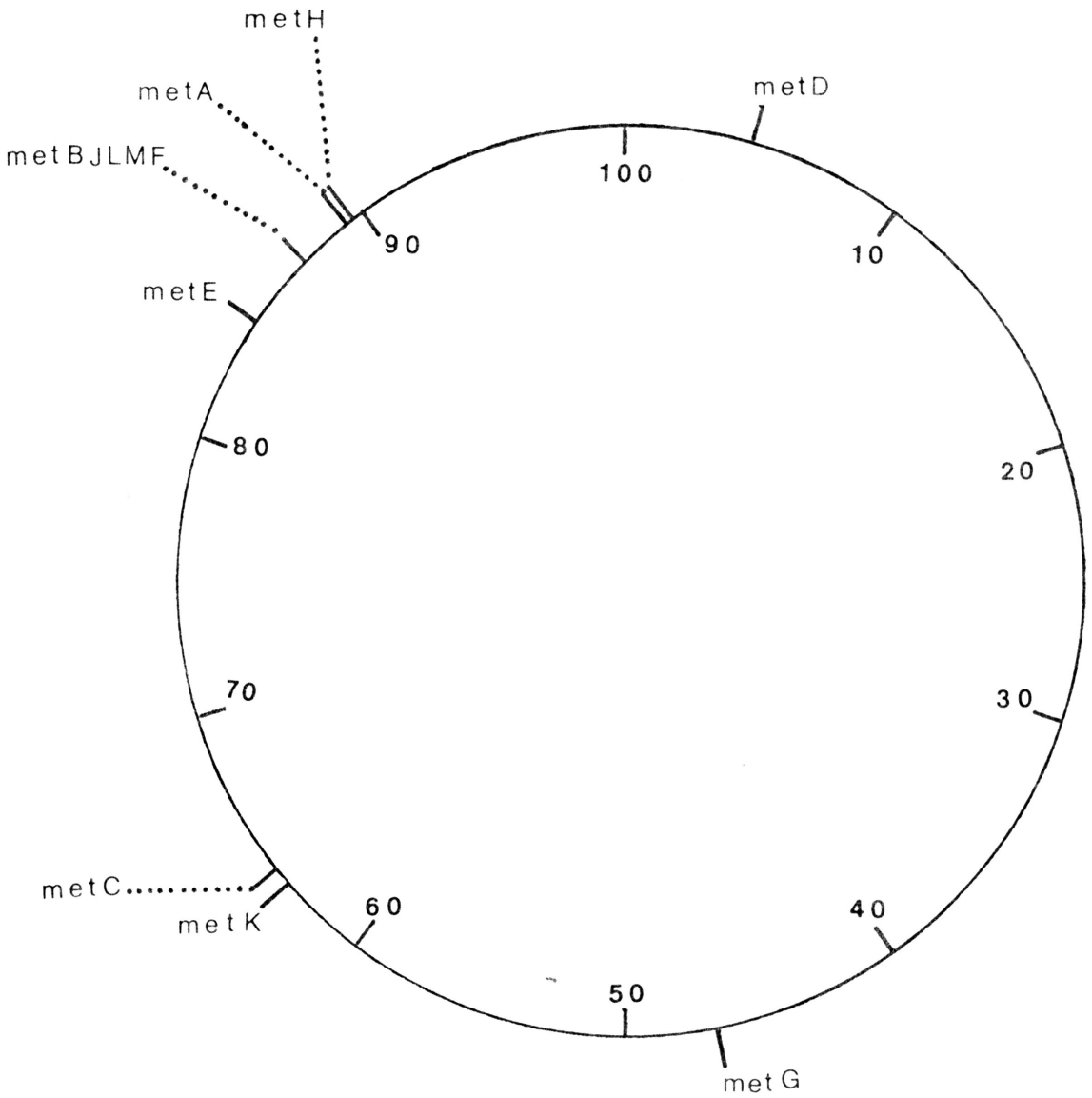


Fig. 1. The positions of the met genes on the recalibrated linkage map of the E. coli chromosome.

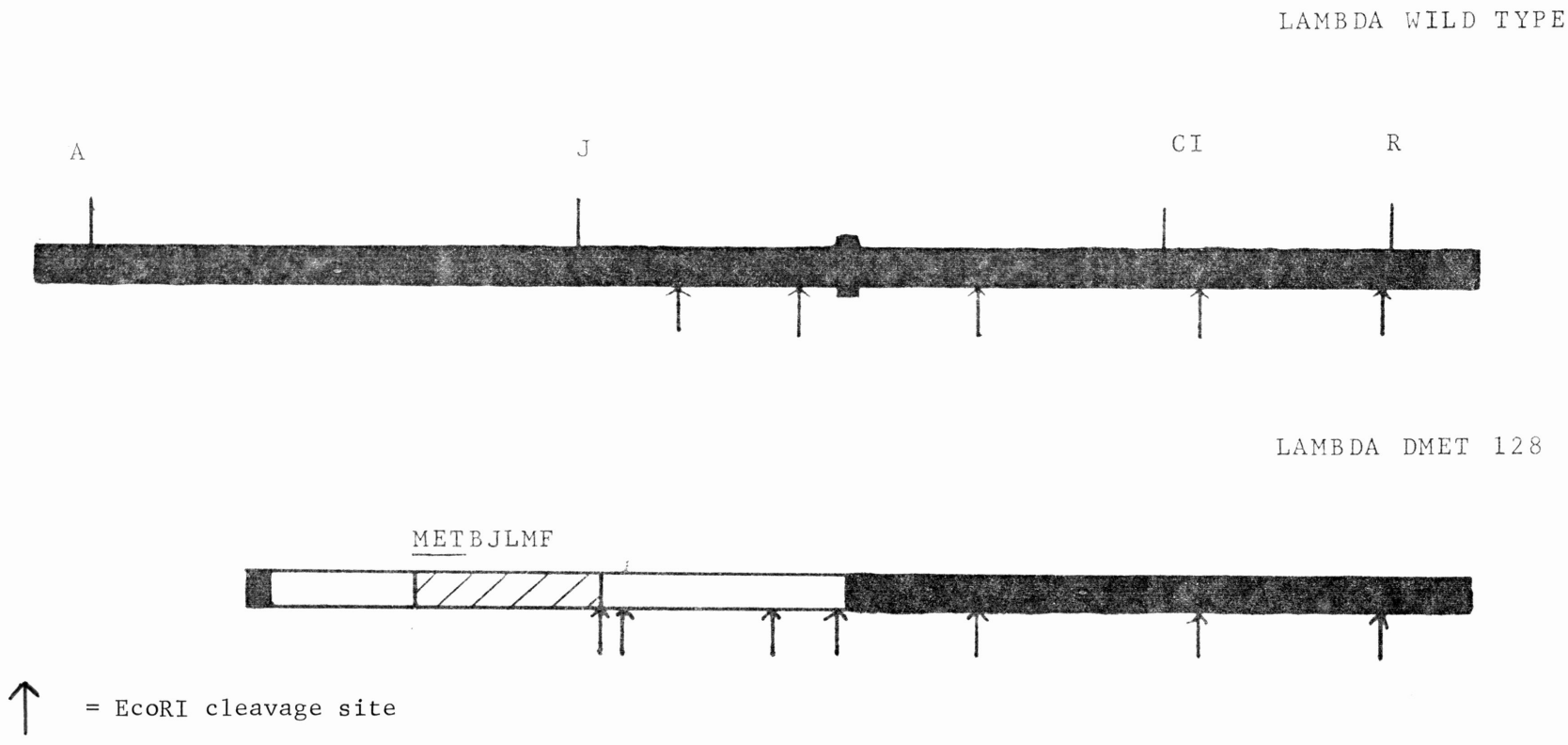


Fig. 2. Genetic maps of lambda wild type and lambda dmet128. The dark regions represent viral DNA; the light regions represent substituted E. coli DNA. EcoRI cleavage sites and some gene locations (letters) are shown.

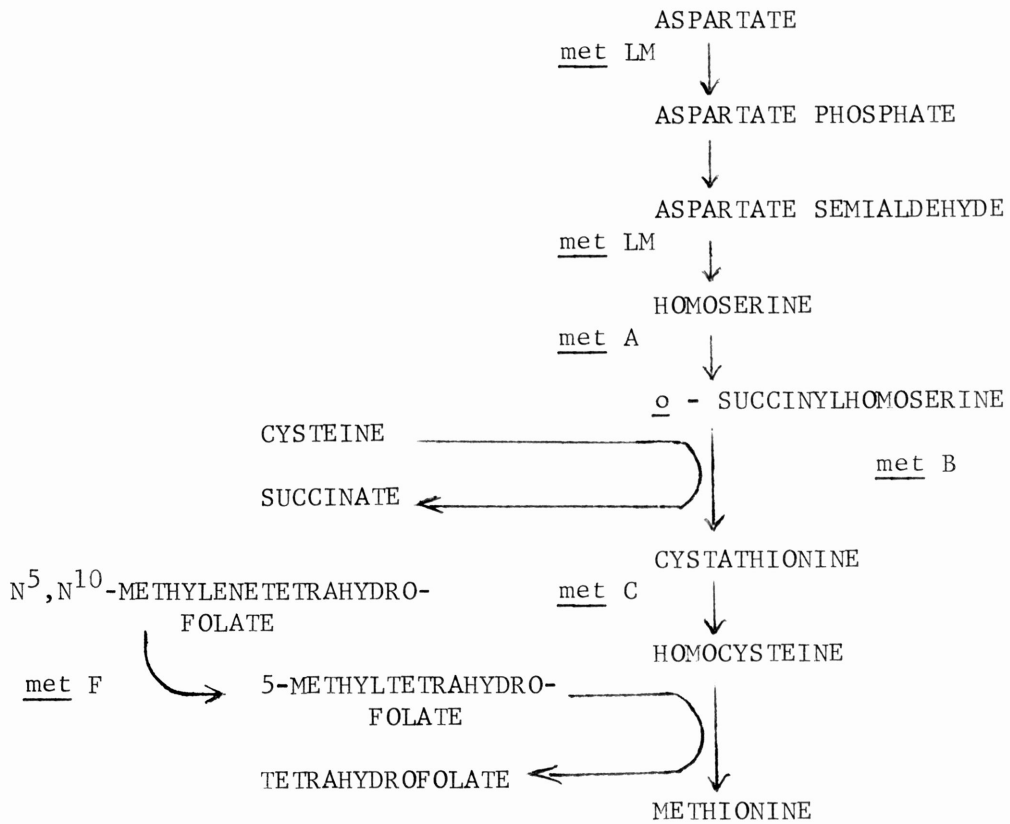


Fig. 3. The methionine biosynthetic pathway in E. coli. The genes responsible for producing the enzymes that perform some of the conversion steps are indicated (met A, met B, etc.). This figure is condensed from Flavin (1975).

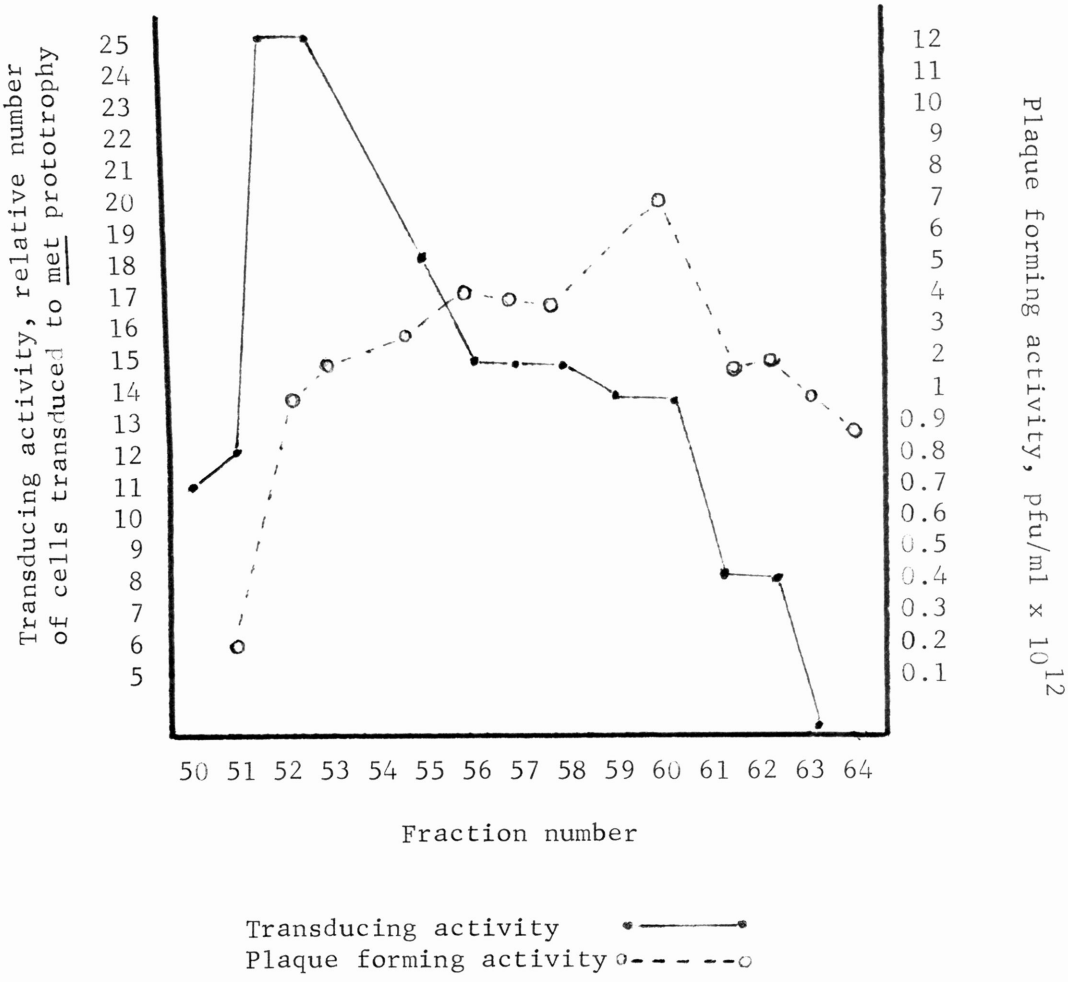
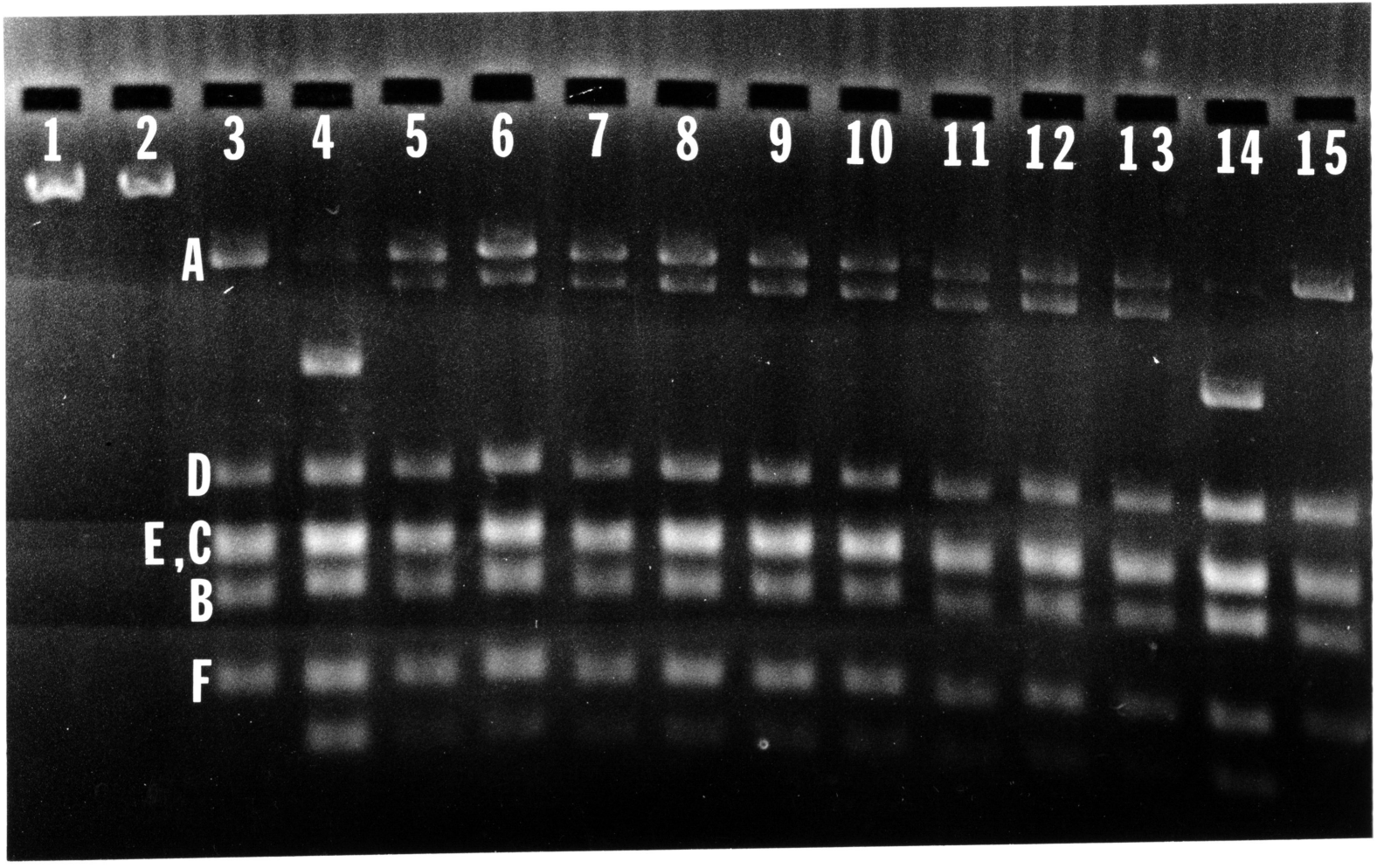


Fig. 4. Analysis of a portion of lambda dmet 128::Tn5 MW77 fractions. Ninety fractions of four drops each were collected from the top to the bottom of a CsCl density gradient (see Text). This portion represents the peak of virion concentration.

Fig. 5. Photograph of agarose gel electrophoresis patterns of lambda dmet128::Tn5 MW77 fractions and controls. See Text for details of preparation.

Patterns no. 1 and 2 are undigested DNA controls. No. 3 is EcoRI digested lambda wild type DNA. The letters refer to the recognized fragments of known molecular weights (Daniels et al., 1980). No. 4 is EcoRI digested lambda dmet128 DNA. Patterns 5 through 13 are the EcoRI digested DNA samples of lambda dmet128::Tn5 MW77 fractions 52, 54 through 60, and 62 respectively. No. 14 is EcoRI digested dmet128 again, and no. 15 is digested wild type.

Notice that in each of the lambda dmet128::Tn5 MW77 fractions the expected topmost band of lambda dmet128 is shifted to a higher molecular weight position (just below the A fragment of wild type). According to the data plotted in Fig. 6, this shift represents 5.5 kilobases of DNA. This is exactly what one would predict from insertion of Tn5 (5.6 kilobases) into this fragment.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
A
D
E,C
B
F

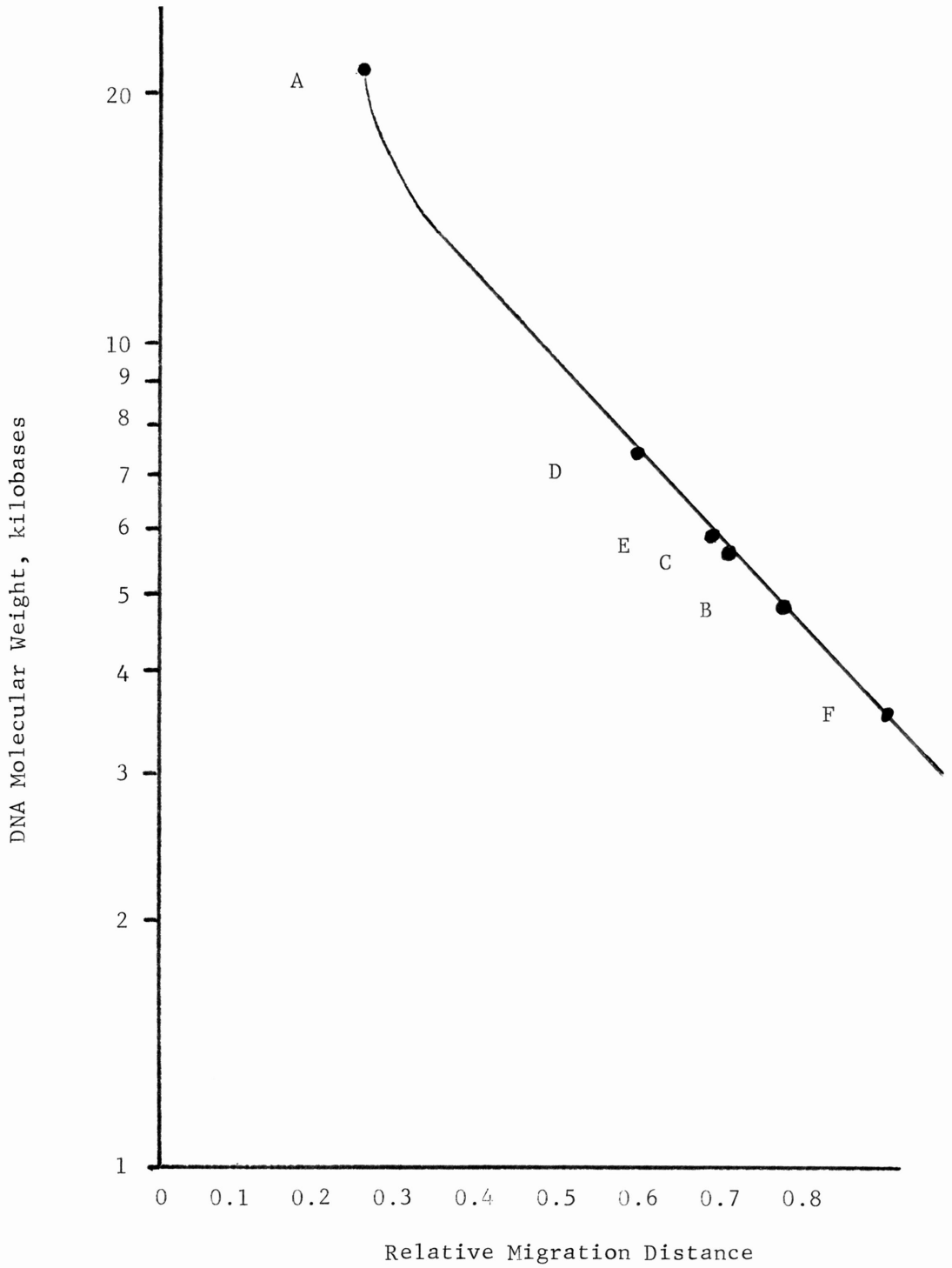


Fig. 6. Molecular weight of DNA fragments versus relative migration distance in an electrophoresis gel (see Text). The letters denote the recognized EcoRI restriction fragments so marked in Fig. 5.

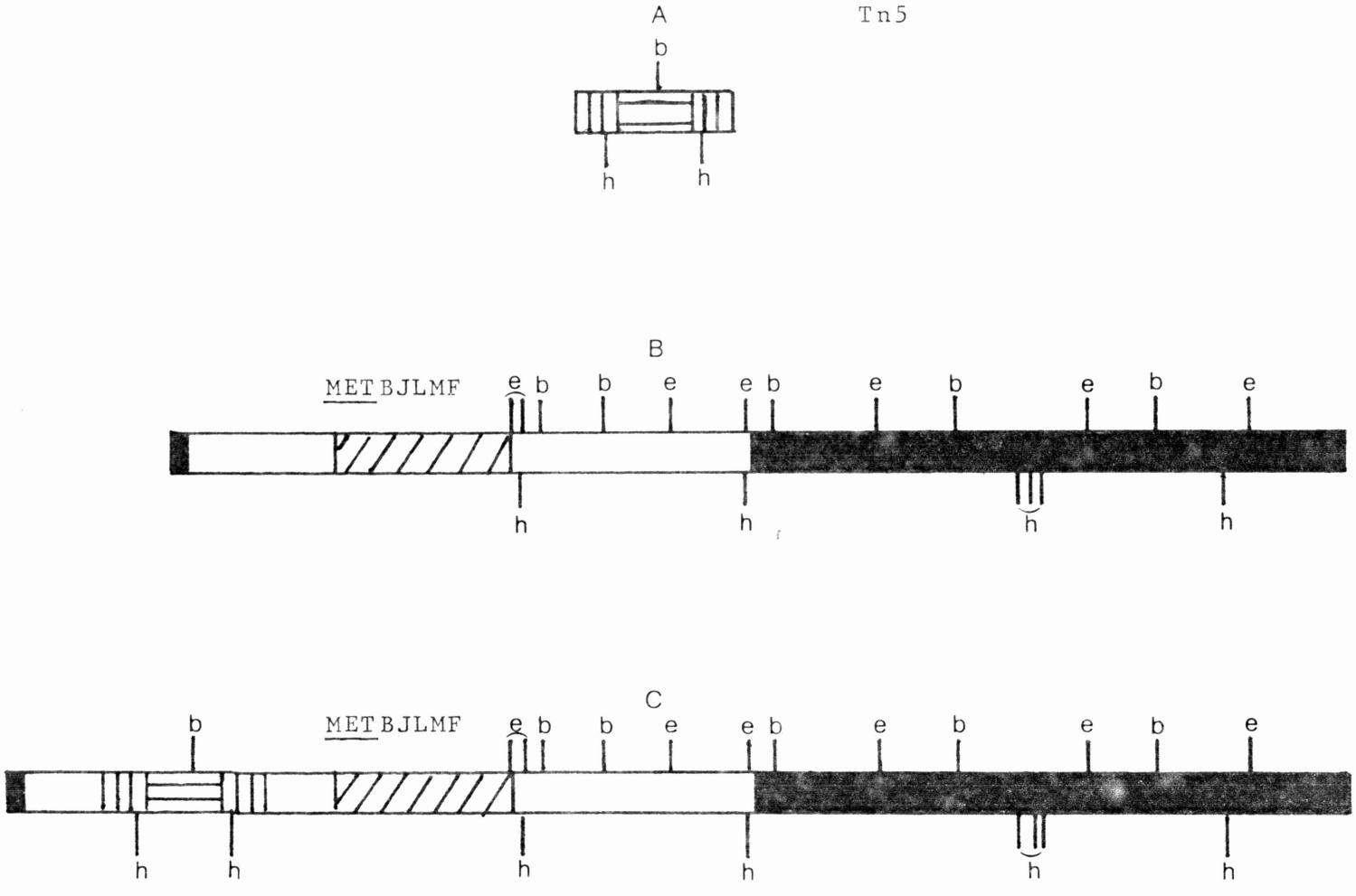


Fig. 7. One possible insertion of the Tn5 element. Letters denote endonuclease cleavage sites: e = EcoRI, h = HindIII, and b = BamHI.