Isolation and Characterization of the *metJB* Transcriptional Locus of *Eschericia coli* K12

by

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DEDICATION

This research is dedicated to the God of Jacob who taught this man called Isreal patience, perseverance, and the meaning of commitment. Lambda can be very much like wives, fathers in law, sheep, and brothers. (Gen. 30-32).

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SUMMARY

Two sets of deletion mutants of lambda pmet100 were isolated by EDTA/heat shock inactivation. A procedure has been developed to quickly screen large numbers of these mutants. The structures of the resultant mutants were studied by an improved plaque assay for the presence of the metB gene in transducing phage and by restriction endonuclease mapping.

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Introduction

The amino acid methionine was discovered in 1922 (reviewed in Mueller, 1961) and its structure was characterized in 1928 (Barger, 1928) Fig 1. By the late 1960's, the role of methionine in three seperate metabolic processes was known. Methionine was found to be an almost universal methylating agent, a precursor to the production of all proteins, and a common intermediate in the synthesis of spermine and spermidine (reviewed in Flavin, 1975). The functions of methionine are similar in both prokaryotes and eukaryotes. Since humans lack the biosynthetic pathway for the production of methionine, it is an essential amino acid in their diet.

Methionine metabolism has been studied in Escherichia coli, Neurospora crassa, plants, and animals. Methionine belongs to the aspartate family of amino acids and its biosynthesis includes common intermediates with lysine and threonine (Flavin, 1975). Mammals lack all but the terminal step of the methionine pathway making this a nutritional requirement for these organisms.

The methionine metabolic pathway is less well studied in plants, but it appers to be similar to the pathways known to exist in fungi and

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Abreviations; DM, Davis-Mingioni; EDTA, ethylenediaminetetracetic acid; kbp, kilobase pairs; LB, Luria Broth; met, Methionine ; PEG, polyethylene glycol; ZRB, Zubay Resuspension Buffer; SAM, S-adenosyl methionine.

This paper was prepared in the form of Gene.

bacteria (reviewed in Giovanelli, 1980).

In prokaryotes, the methionine pathway is held in common with that of lysine and threonine through two enzymatically controlled reactions converting aspatrate to aspatate-B-semialdehyde. This semialdehyde branch point leads by a single reaction to homoserine, where the pathway divides to either methionine or threonine. Methionine is produced by four more steps unique to methionine biosynthesis (Bender, 1975) Fig 2.

The regulation of the methionine production in prokaryotic systems is not well understood at this time. In the coliform bacterium E. coli, it is known that methionine biosynthesis is orgnized as a coordinated regulon of at least ten gene units (reviewed in Flavin, 1975). The two known patterns of regulation of methionine biosynthesis consist of: 1) feedback inhibition of the enzyme homoserine transuccinylase (effectors are methionine and S-adenosyl methionine) which is the product of the metA gene, and 2) general inhibition of the expression of all the gene products of the methionine regulon presumably at the level of transcription by the gene products of metJ and metK (reviewed in Flavin, 1975). The product of the gene metK is S-adenosyl methionine (SAM) synthetase while the gene product of the metJ gene has yet to be identified though an apprepressor protein acivated by a SAM derivative is suspected (reviewed in Flavin, 1975; Liljestrand-Golden and Johnson, 1983). The metJ gene is located in the metJBLMF gene cluster at the 88 min region of the recombination map of the E. coli chromosome (Bachman and Low, 1980).

Using the Shimada technique (Shimada et al., 1972), several lambda type transducing phage have been constructed which carry varrying amounts

of the metJBLMF gene cluster of the E. coli chromosome (Johnson, 1977; Liljestrand-Golden and Johnson, 1983). One specific transducing phage designated lambda pmet100, carries in its left arm a 6.4 kilobase pairs (kbp) unit of bacterial DNA. This bacterial DNA contains a 3 kbp region with a functional copy of both the metJ and metB genes as determined by transductional tests and enzyme activity assays (Liljestrand-Golden and Johnson, 1983) Fig 3.

The goals of this project were to isolate and characterize mutants of the lambda pmet100 transducing phage which contain deletions of DNA within the metJB region and to use the deletion types to map by function and transcriptional organization these two genes. The technique employed to isolate deletion mutants was that of EDTA/heat shock inactivation, which has been previouly described (Parkinson and Huskey, 1971). The process has been shown to select for and amplify the frequencey of the naturaly occuring deletion type phage in lysates through several cycles of EDTA/heat shock. The process depends upon the fact that lambiod phage are suseptable to EDTA/heat shock inactivation in an inverse relationship to the total DNA content of the phage particle. Phage with normal or larger DNA compliments will be inactivated more rapidly by the chelating action of EDTA than phage with smaller genomes

To characterize the deletion mutants discovered during EDTA/heat shock selection, a plaque assay previously described for the detection of the prescence or absence of a functional metB gene in a transducing phage (Johnson and Liljestrand, 1983) was perfected.

MATERIALS AND METHODS

(a) **Strains**

All the bacterial and viral strains used or constructed for this study are listed in Table 1.

(b) **Media**

The media used in viral growth and plauqe assays have been described previously (Johnson et al., 1977). The make up of the agar media used for the fast DNA plate lysates was obtained from Dr. Terry Thomas (personal communication) and is described in the procedure.

(c) **Enzymes and Chemicals**

The Sma1 and BamH1 restriction endonucleases were obtained from BRL. All other chemicals were of reagent grade and were procured from standard scources.

(d) **Halo Assay**

The metB halo plaque assay used in this study was a modified version of the assay previously described (Johnson and Liljestrand, 1983). This assay consisted of plating 50-100 pfu of the phage in question on a lawn of metB$^-$J$^-$ cells (JJ131 Su-3). These cells were grown for six to eight hours in a DM minimal medium suplimented with 0.5% maltose and 4.0mM L-methionine and then washed twice in DM minimal medium. An appropriate volume of the phage was mixed with 2.5 ml DM top agar and 0.5 ml of the cells. The mixture was poured onto a DM minimal plate after a 10 min absorption period. After hardening of the agar, a second overlay consisting of 0.5 ml cells, 0.015 ml of a 0.02 M L-methionine solution, and 2.5 ml of the DM top agar was added over the plates. The preparations were incubated at 42^o C for eight hours and then at 32^o C overnight. The assay was scored as positive for the presence of a functional metB gene if the plaques formed produced a halo

of bacterial growth around the periphery. A typical halo plate is depicted in Fig. 4 and shows the four phenotypes observed: haloed (A), non-haloed (B),), specked (C), and super haloed(D).

(e) **EDTA/Heat Shock Inactivation**

The EDTA heat shock inactivation proceedure was used to select and amplify mutant deletion types. This series of experiments was performed upon four lysates (C, D, E, and F) of lambda pmet100 obtained from clonal lysogens of the bacerial cell JJ116. Lysates were prepared from cultures of each lysogen. and the resulting phage were tested for their metB homgeneity by the halo plaque assay (greater than 99.9% of initial plaques produced halos indicating genotypic uniformity). A sample of each lysate was then diluted 1/100 into prewarmed 0.01 M EDTA at either 42^{o} C or 40^{o} C and incubated for a period of 15 min at temperature. Each cycle of inactivation was designed to produce a one to two $\log^{10}$ drop in the titer of the lysate as determined by the time of incubation in the inactivation solution. The inactivated material was then diluted 1/10 into a pre-chilled stop buffer consisting of 0.1 M Tris pH 7.5, 0.1 M magnesium sulfate, 0.01 M calcium chloride, and 0.01% gelatin. The stoped material was then titered with RW262 host cells. Simultaneously, the next generation of phage was produced by plating about $1X10^{6}$ phage on fresh LB lambda plates with 5.0 mls of half strength LB lambda top agar and 0.5 mls host cells. These lysates were then incubated at 37^{o} C. for ten to twelve hours, chloroformed, and the phage harvested by centrifuging out the cell debris. This produced for the next generation, 4 to 6 mls of phage per lysate at a concentration of $1X10^{8}$ to $1X10^{9}$ phage/ml.

After each cycle of EDTA/heat shock inactivation, the plate lysates and stop tubes were titered and the lysates tested for deletion types using the halo plaque assay. Each clonal isolate was inactivated at 42^{o} C. and 40^{o} C. by the EDTA heat shock process for 3 cycles of inactivation.

Possible deletion types were chosen from the halo plaque assay plates of each inactivation and the plaques removed for isolation into 2 mls of ZRB. The pickings were then chlorophormed, titered on RW262 host cells, and tested for homogineity using the halo plaque assay. Pure pickings (99.9% haloed or non-haloed plaques designated purity) were amplified in titer from about $1x10^{6}$ phage per milliter to about $1x10^{9}$ phage per milliter using the plate lysate procedure described above.

(f) **Fast DNA preparation**

Once deletions had been isolated, purified, and amplified, it was then possible to prepare DNA samples for electrophoretic gel comparison using the mini lysate proceedure of Dr. Terry Thomas (personal communication). In this proceedure the deletion phage were plated on LB plates and top agar suplemented only with magnesium sulfate (0.01M) using either JJ131 Su-3 or RW262 cells that had been grown in LB lambda medium for four to six hours. This plating was performed at a phage concentration which would to achieve an almost confluent series of plaques. The plates were incubated at 42^{o} C. for eight hours to allow for plaque formation. After this period, the plates were chilled at 4^{o} C. to harden the agar and then 4.0 mls of sterile 0.01 M Tris 0.01 M magnesium sulfate pH 7.3 was added and gently swirled with 4-5 drops of chloroform over the surface of the plates. This preparation was then

left overnight at 4^{o} C.

To harvest the phage DNA from the mini plate lysates, the liquid on the surface of the plates was swirled to pick up the phage on the bacterial lawn and then decanted into sterile tubes. Without delay, the phage were dispensed in 0.5 ml samples in 1.5 ml microfuge tubes and treated with 50 ul of a solution containg 2M tris and 0.2M EDTA pH 8.0. Also added was 5.0 ul of diethylpyrocarbonate and 10 ul of a 10% sodium dodecyl sulfate solution. This mixture was incubated in a water bath for 10 min at 70^{o} C. Subsequently, 32 ul of 8M sodium acetate was added to each sample and the material was allowed to precipitate for 30 min at 0^{o} C. The samples were then cetrifuged at 12,000 x g for 15 min, the supernatants were saved, and each was treated with twice its volume of 100% ethanol. Again the samples were centrifuged and the pellets washed three times with 70% ethanol. Finally, the precipitates were dried under a vacuum for one hour to remove residual ethanol.

(g) **DNA Digestion and Electrophoresis**

To compare the various genotypes and to map the deletion sites, the DNA from the deletion types was first digested with Sma1 type endonuclease and then seperate samples were digested with either BamH1 or HindIII. Most of the digestions were carried out in solutions containing 20 ul of the DNA, 5 ul of the respective enzyme buffer, and usually 1.0 ul of the enzyme itself. These digestion solutions were allowed to incubate with very gentle shaking for 4-8 hours at 37^{o} C depending upon the relative activity of each enzyme preparation.

The specific digestion buffers for each enzyme were prepared as suggested in the 1982 BRL catalog. The stop solution for all of the

enzyme digestions consisted of a final concentration of 20mM EDTA, 20% glycerol, 0.025% bromphenol blue, 0.1% SDS, and 0.4% Sea-plaque low-temperature agarose.

Stopped DNA digests were heated for three min at 65^{o} C and then loaded into 2mm wells at the head of a 1.0% agarose slab. The electrode buffer consisted of Tris, sodiumacetate, and EDTA pH 7.8 as previously described (McDonell et al., 1977).

Final visualization of the DNA in the agarose gels was accomplished by staining the gels for 15 min in 250 mls of distilled water containing 0.5 mls of a 1 mg/ml ethinium bromide stock solution. Subsequent destaining used 250 mls of distilled water for 15 min. The gels were then immediately photographed under u.v. light and the negatives developed as previously described (Liljestrand-Golden and Johnson, 1983).

(h) **Large Scale Lysates and Final DNA Preparations**

The large scale lysate process used to prepare one to two mls of clean DNA that could be used to accurately determine the electrophoretic pattern of the digested deletion types has been previously described (Maniatis et al., 1982). This process consisted of infecting a 500 ml TBM culture of RW262 cells at an optical density (550 nm) of 0.10 with approxamately 1X10^{6} phage. These cultures were induced at 42^{o} C for 15 min and then incubated at 39^{o} C for up to 5.5 hours with vigorous shaking. After this time, or upon visual lysis of the cell culture, 5 ml of chloroform was added and the cultures were allowed to stand at room temperature for 10 min. The chloroform was then removed, the solutions were centrifuged at 11,000xg for 10 min, 10% w/v of solid PEG was added with 29.2 of g sodium chloride, and then the solutions were incubated

overnight at 4^{o} C. The precipitated phage were then pelleted by centrifugation at 11,000xg for 10 min and gently resuspended in 8 ml of the ZRB. The PEG was then extracted with an equal volume of chloroform, and the clarified phage solution was purified by centrifugation in a 1.4/1.5/1.6 g/cc cesium chloride gradient. The phage band was removed from the 1.5/ 1.6 interface and dialized against the ZRB and then 0.1M sodium phosphate buffer at pH 7.1.

The DNA was released from the above phage preparations by two extractions with equilibrated phenol (0.1 M Tris pH 7.1) and 0.4% SDS. The DNA was then dialized against 0.01 EDTA pH 8.0 and then 0.01M Tris pH8.0 to remove the phenol and EDTA.

Preparations of DNA were digested with Sma1 and BamH1 restriction enzymes. The usual volumes used were: 0.06ml DNA sample, 0.02ml digestion buffer (specific for the enzyme), 0.02ml distiled water, and 0.005 ml of the enzyme solution. These mixtures were incubated at 37^{o} for 4 to 6 hours and then subjected to elecrophoretic seperation as stated previously.

(h) **metJ Assay**

The assay used to determine the metJ character of the deletion types consisted of determining the relative activity of the metC gene product (beta cystathionase) whose expression is repressed in the presence of a functional metJ gene. The beta-cystathionase assay (Green et al., 1973) was performed on fresh, overnight cultures of lysogens of the respective deletion mutants grown in LB broth. 0.25 ml samples were taken and resuspended in a 1.0 mg/ml solution of egg white lysozyme, 0.02M Tris and 0.001M EDTA pH 7.6. These samples were incubated at room temperature for

15 min and then subjected to three cycles of freezing and thawing in liquid nitrogen. The cell extracts were then mixed with 0.1 ml of the reaction mix consisting of: 5 parts 0.002 M Ellmans Reagent in 0.2 M potassium phosphate buffer pH 7.42, 2 parts 0.005 M l cystathionine, 1 part 0.00125 M magnesium sulfate, and 1 part 0.6 M potassium phosphate buffer at pH 7.42. This mixture was allowed to incubate at room temperature for 30 min at which time the strength of the developing yellow color relative to metJ positive and metJ negative cells was recorded visually. The strong yellow color was represenative of active beta cystathionase and therefore of an inactive metJ allele.

Results and Discussion

The system used to isolate and characterize deletion mutants of lambda pmet100 is outlined in Fig 5. This procedure consisted of selecting for and amplifying the frequency of naturally occuring mutants that have deletions within their genomes by the process of EDTA/heat shock inactivation (Parkinson and Huskey, 1971). Many plaques were picked by phenotype from halo plaque assay plates, and then rapid, but crude, plate lysates were prepared from which samples of DNA could be extracted, digested with restriction endonucleases, and then subjected to electrophoretic seperation. These steps constitute the presumptive deletion tests. Large scale lysates were then made of identified deletion mutants for more precise characterization by electrophoretic seperation and beta cystathionase assay.

The inactivation data obtained from the EDTA/heat shock process is

presented in Table 2. The data consists halo plaque assays performed upon the phage surviving each of three cycles of inactivation at two different temperatures. Because of the marked increase in the frequencey of deletion mutant types observed (as measured by the increase in the frequency of nonhaloed plaques), it can be concluded from this data that the EDTA/heat shock treatment does act as an enrichement process for deletion mutants. Upon the basis of the later maping data presented later, it was also observed that the inactivation performed at the higher temperature did on the avarage result in larger deletions.

The maping data resulting from the two series of inactivations is presented in the Fig.s 6, 7, 8, of rapid DNA preparations and Tables 3, 4, and 5. Fig. 6 depicts the Sma1 restriction pattern after electrophoretic seperation of the restriction fragments and is representative of the presumptive screening of the deletion types. Table 3 summarizes the data obtained from analysis of these rapid DNA preparations. It can be seen that the gel patterns resulting from the rapid DNA preparations give only an approximation of the number, identity, and molecular weight of restriction fragments. The strength of this procedure lies not in its absolute accuracy but in the rapidity by which mutants can be screened.

The systematic mapping of the deletion mutants obtained from these efforts utilized the known molecular weights of the lambda wild type and lambda pmet100 fragments obtained by electrophoresis after endonuclease digestion with the Sma1 and BamH1 enzymes(Liljestrand and Johnson, 1983) (Fig. 9). The Sma1 restriction enzyme is especially usefull in this respect. The lambda wild type used in this study produced, after Sma1

digestion, four fragments of molecular weights 19.6, 12.3, 8.7, and 8.4 kbp (bands A, B, C, and D) while lambda pmet100 omitted the 12.3 fragment and added three new bacterial bands of molecular weights 5.5, 4.4, and 2.9 kbp (1, 3 and 2). The order of these fragments within the viral genome is shown These enzyme cut patterns results in the convenient seperation of the bacterial DNA fragments from the viral fragments. Sma1 banding patterns of the deletion types were compared with the above standards to determine the respective molecular weights. With this information as well as the metB and metJ phenotypes it was then possible to deduct the size and general position of the missing DNA as shown in Table 3.

The mapping procedure will be illustrated using the deletion mutant EH 201 (Figs 6 and 7). The Sma1 restriction pattern of this deletion type omitted the 12.3 kbp viral fragment of the wild type, but retained the viral 19.6, 8.7, and 8.4 bands plus a 4.4 kbp fragment similar to the 4.4 fragment of lambda pmet100. The only other band that EH 201 exhibited was a new 4.8 kbp fragment, omitting both the 5.5 and 2.9 kbp bands of lambda pmet100. It was also known that this deletion type showed the $metB_+$ phenotype as determined by the halo plaque assay and summarized in Table 5. From this data it was deduced that the new band was a fusion of the 5.5 and 2.9 kbp fragments of lambda pmet100 by the deletion of the Sma1 cut site between these two fragments. A non-deletion fusion of these two bands should have produced a fragment of 8.4 kbp (5.5 + 2.9), so the deletion must remove approximately 3.6 kbp of bacterial DNA (8.4 - 4.8). Because the metB gene and associated bacterial and viral DNA is known to occupy at least 3.772 kbp of the left side of the 5.5 kbp

fragment and since EH 201 is a metB$^+$ phage, it was concluded that no more than 1.7 (5.5 -3.8) kbp of the 5.5 kbp fragment could have been deleted. This mean that at least 1.9 (3.6 - 1.7) of the 2.9 kbp fragment must be deleted. If one were to assumed that metJ were present as was determined later by beta-cystathionase assay, then at least 4.42 kbp of the 5.5 kbp fragment must be present (Liljestrand and Johnson, 1983). This assumption would mean that 1.1 kbp (5.5 - 4.4) of the 5.5 kbp fragment had to be deleted and that 2.5 kbp of the 2.9 kbp frgment would be missing.

The resulting map of EH 201 is shown in Fig. 8 and suggests that the metB gene has been isolated on a relatively short fragment of DNA. This 4.8 kbp fragment certanly holds 2.90 kbp of bacterial DNA which defines the metB and metJ genes. This fragment could be very usefull in cloning studies and in studies of the transcriptional organization of the metJ gene.

The maps obtained for the other deletion types are depicted in Fig 8 and the calculated deletion sizes and genotypes are presented in Tabel 5.

During the halo plating of two of the deletion types, a unique morphology of halo plaque described as superhaloed was observed (Fig. 4.). The deletion types CH 100 and CH 104 consistantly produced plaques with larger and stronger halos than those of lambda pmet100 or of any of the other deletion types. As the halo assay was designed, the overhaloed morphology could be directly related to hightened methionine production. Both phage scored as metB$^+$J$^+$ and although they originated from the same lysogen strain, CH 100 was isolated from the lysate inactivated at 42^o C while CH 204 was isolated from a seperate lysate inactivated at 40^o C.

Upon electrophoretic banding and subsequent maping (Fig. 8), the mutant CH 100 was shown to delete a large section of the right most bacterial DNA as well as a segment of viral DNA containing one of the viral SmaI cut sites in the right arm. The presence of a transcriptional terminator sequence has been postulated for this deleted region to explain the CH 100 deletion plaque morphology. The absence of this terminator would allow the viral PL promoter to express the bacterial genes from a transcriptional unit in the right arm of the viral chromosome (reviewed in Szybalsky, 1977). Since the viral promoter is not responsive to metJ repression, the cells infected by CH 100 are presumably producing large quantities of the metB gene product. The data on CH100 also supports the proposition that the metB gene is transcribed from right to left in the bacterial cell (Liljestrand and Johnson, 1983) since the viral promoter expressing metB in CH 100 would promote transcription of the metB gene only in this orientation (right to left on the lambda CH 100 map).

This study of the deletion mutants of the transducing phage lambda pmet100 has provided data concerning and techniques for manipulations of the E. coli genes of the metJB locus. The halo assay for phage with metB activity has also been refined and proven consistent. The proceedures developed during this study include the integration of the EDTA/heat shock technique with the halo plaque assay and the rapid plate lysate technique for DNA preparation to quickly isolate and characterize deletion mutants of transducing phage carrying the metJB locus.

The data generated by this study has also established deletion frequencies and confirmed the efficacy of the EDTA/heat shock procedure

for selecting such mutants. Also, two sets of deletion mutants have been isolated and mapped, a small fragment has been identified that carries the isolated metBJ genes, and supporting data has been generated on the ploarity and independence of metB expression.

Work still needs to proceede on mapping of the precise location and fine structure of the present deletion mutants via S_1 nuclease digestion (Maniatis et al., 1982). Enzymatic studies need to be done to confirm the metJ activity of the existing viral lysogens carrying deletion types. Also, further isolation and subsequent characterization of deletion types obtained from EDTA/ heat shock treatment at different temperatures needs to be performed to obtain a wider variety of deletion types, in partiular isolates which carry either metB or metJ but not both genes. Recently, a single isolate of this type which carries the metB but not the metJ gene has been isolated and characterized (Liljestrand and Johnson, 1983). It is interesting that none of the deletions characterized in this study were of this type. In addition, the presumed effects of the superhalo phenotype on metB expression must be confirmed by monitoring the metB expression during lytic growth of this phage and appropriate controls (studies in progress).

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APPENDIX

TABLE 1

LIST OF BACTERIAL AND BACERIOPHAGE STRAINS

STRAIN	RELEVENT GENOTYPE	SOURCE
BACTERIAL:		
JJ116	PROTOTROPH	JOHNSON, ET AL., (1977)
JJ118	METJ184	" " "
JJ119	METJAM185	KRUGER, ET AL., (1981)
JJ100	$METJ^{+}$, METB1, $METL^{+}$, $METF^{+}$	JOHNSON, ET AL., (1977)
JJ131	METJ184, METB1, $METL^{+}$, $METF^{+}$	" " "
RW262	TONA, SU2, MAL	MAZAITIS, ET AL., (1976)
JJ131 Su3	METJ184, METB1, $METL^{+}$, $METF^{+}$, SU3	Liljestrand and Johnson, (1983)
BACTERIOPHAGE:		
LAMBDA WILD TYPE	CI indlts857Sam7	SANGER ET AL., (1982)
PMET100	$METJ^{+}$, $METB^{+}$	LILJESTRAND AND JOHNSON, (1983)

TABLE 2

FREQUENCY OF NON HALOED MUTANTS THROUGH

THREE CYCLES OF EDTA/HEAT SHOCK INACTIVATION

Lysate	Percentage of Non Haloed Plaques in the Viral Population[a] Per Cycle of EDTA/Heat Shock[b]					
	Cycle 1		Cycle 2		Cycle 3	
	42° C	40° C	42° C	40° C	42° C	40° C
C	0.8%	0.0%	16.9%	0.0%	100.0%	ND[c]
D	12.1%	0.0	4.6%	0.0%	95.4%	5.2%
E	13.7%	0.0%	25.4%	0.0%	97.1%	5.6%
F	12.2%	0.0%	12.2%	0.0%	ND[c]	18.6%

a Lysates were prepared from clonal isolates of a lambda pmet100 Lysogen of strain JJ116.

b Inactivations were performed in 0.01 M EDTA pH 8.1 at either 42° C or 40° C.

c ND denotes samples not developed.

TABLE 5

DELETION MUTANT CHARACTERISTICS

DELETION TYPE	TEMPERATURE OF ISOLATION	RELEVENT GENOTYPES	FRAGMENTS FUSED	DELETION LENGTH (KBP)
CN104	42	B^-, J^-	1, 2, & 3	8.00
CN105	42	B^-, J^-	1, 2, & 3	8.00
CN106	42	B^-, J^-	1, 2, & 3	8.00
CH100	42	B^+, J^-	2, 3, & C	6.50
DH101	42	B^+, J^+	2, & 3	3.18
EH100	42	B^+, J^+	1, & 2	3.30
CN200	40	B^-, J^-	1, 2, & 3	5.15
EH201	40	B^+, J	1, & 2	4.8

1. These experiments were performed at 42^O C or at 40^O C.

2. These mutants appeared identical in all respects.

Fig. 1 shows the structure of methionine.

THE STRUCTURE OF METHIONINE

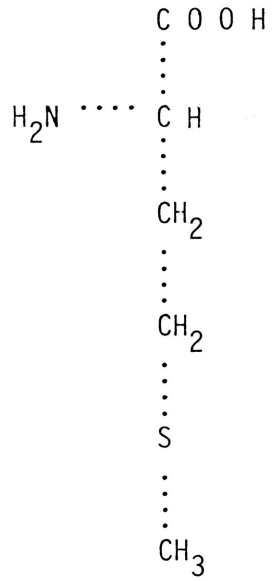


Fig. 2 outlines the methionine biosynthetic pathway with the associated genes and enzymes for each step.

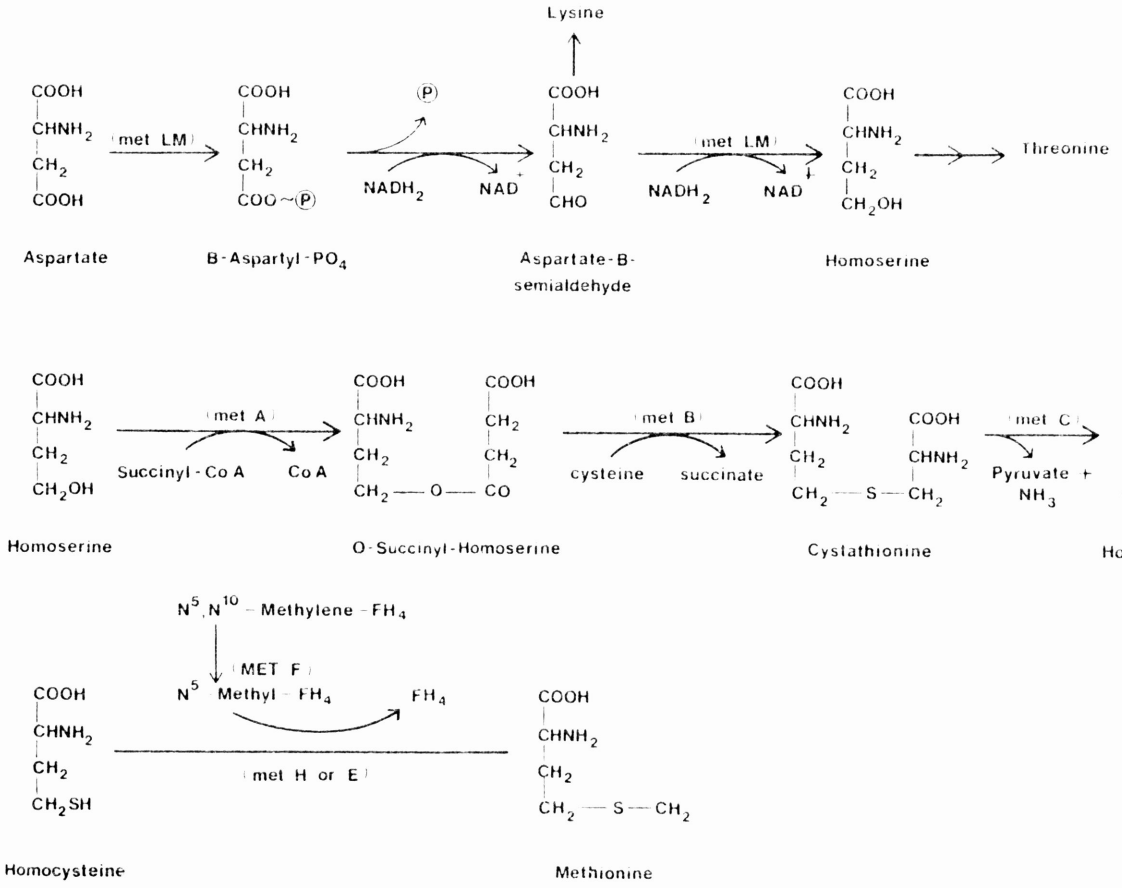

Lysine
COOH
CHNH2
CH2
COOH
(met LM)
COOH
CHNH2
CH2
COO~(P)
(P)
NADH2
NAD+
COOH
CHNH2
CH2
CHO
(met LM)
NADH2
NAD+
COOH
CHNH2
CH2
CH2OH
Threonine
Aspartate
B-Aspartyl-PO4
Aspartate-B-semialdehyde
Homoserine
COOH
CHNH2
CH2
CH2OH
(met A)
Succinyl-Co A
Co A
COOH
CHNH2
CH2
CH2 — O — CO
COOH
CH2
CH2
(met B)
cysteine
succinate
COOH
CHNH2
CH2
CH2 — S — CH2
COOH
CHNH2
CH2
(met C)
Pyruvate + NH3
COOH
CHNH2
CH2
CH2SH
Homoserine
O-Succinyl-Homoserine
Cystathionine
Homocysteine
N5,N10 - Methylene - FH4
(MET F)
N5 Methyl - FH4
FH4
COOH
CHNH2
CH2
CH2SH
(met H or E)
COOH
CHNH2
CH2
CH2 — S — CH2
Homocysteine
Methionine

Fig. 3 is a structural map of the lambda p*met* 100 chromosome compared to that of lambda wild type.

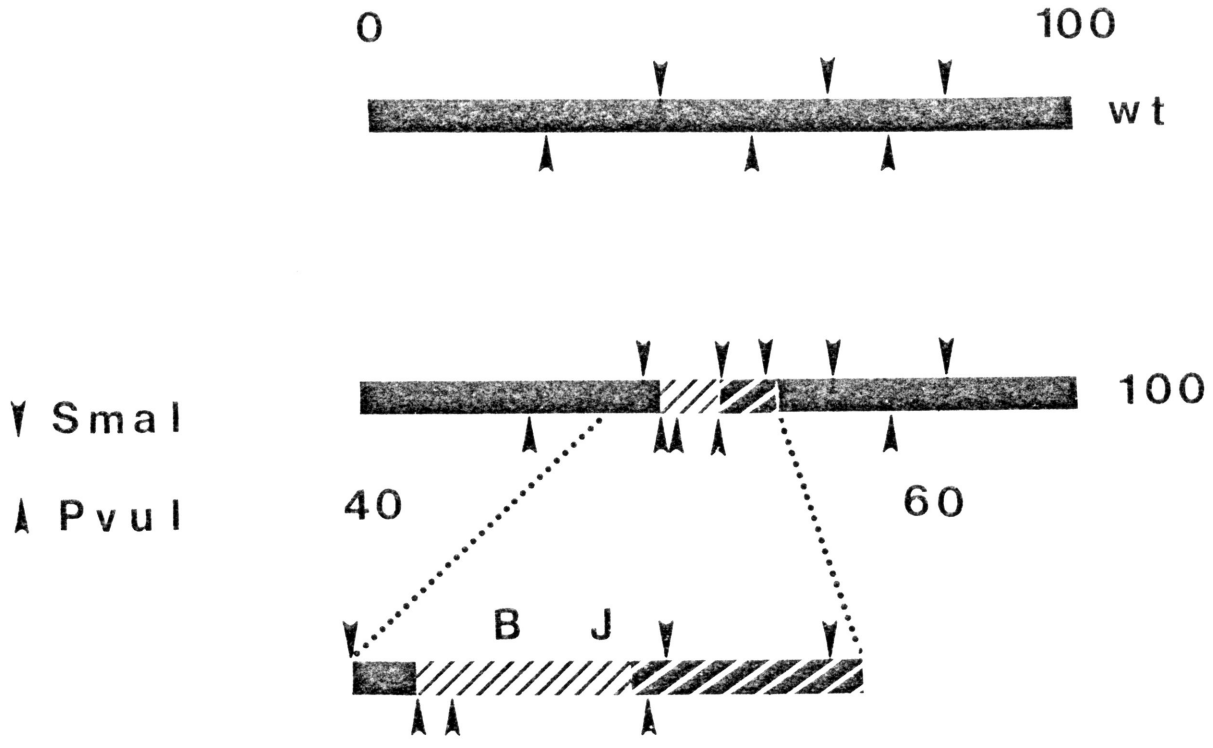

0
100
wt
Sma I
Pvu I
100
40
60
B
J

Fig.4 shows a typical halo plaque assay plate illustrating the four morphologies.

a
b
c
d

Fig. 5 is an outline of the procedures followed in this study.

OUTLINE OF DELETION TYPE

ISOLATION AND CHARACTERIZATION

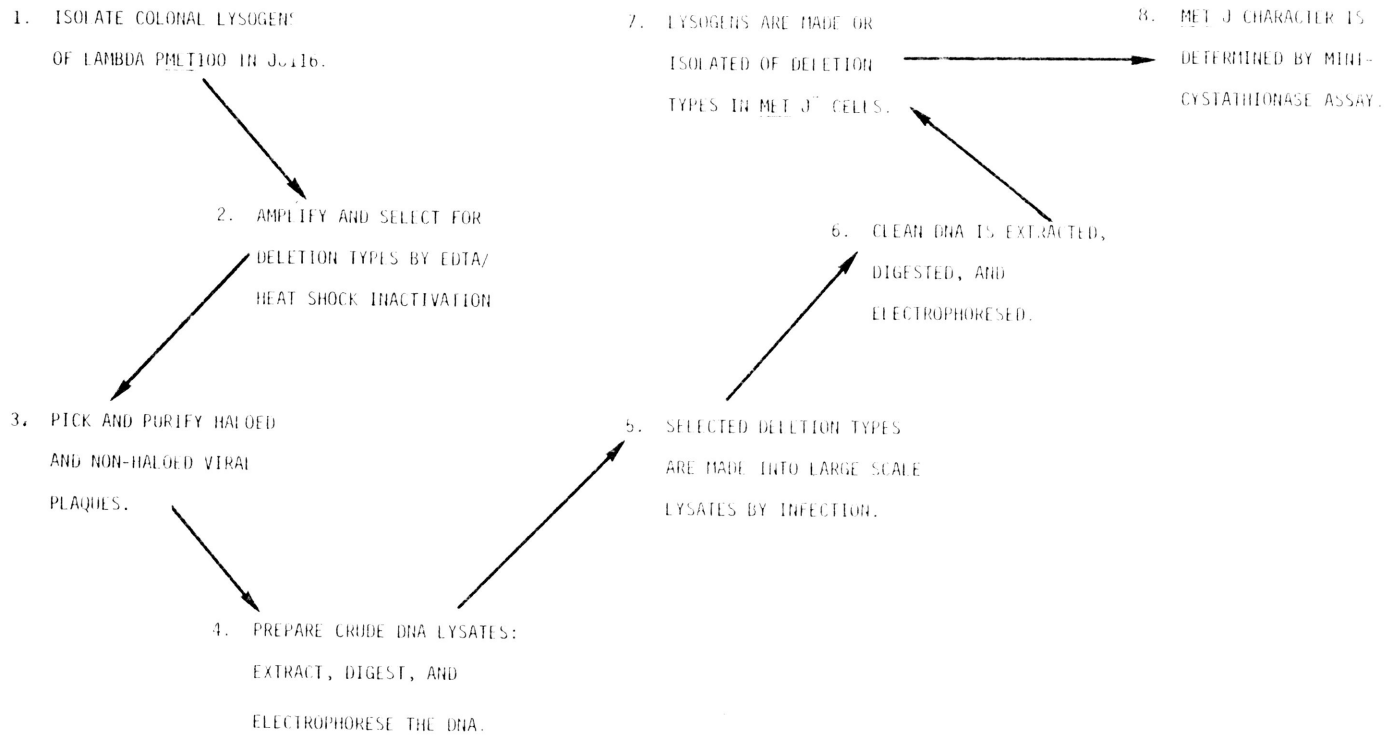


Fig. 6 is a represenative electrophoretic gel agter the rapid plate lysate DNA preparation. This shows the paterns obtained from the isolates in Table 3.

Fig. 6 is a represenative electrophoretic gel after the rapid plate lysate DNA preparation. This shows the paterns obtained from the isolates listed in Table 3.

Figure 6

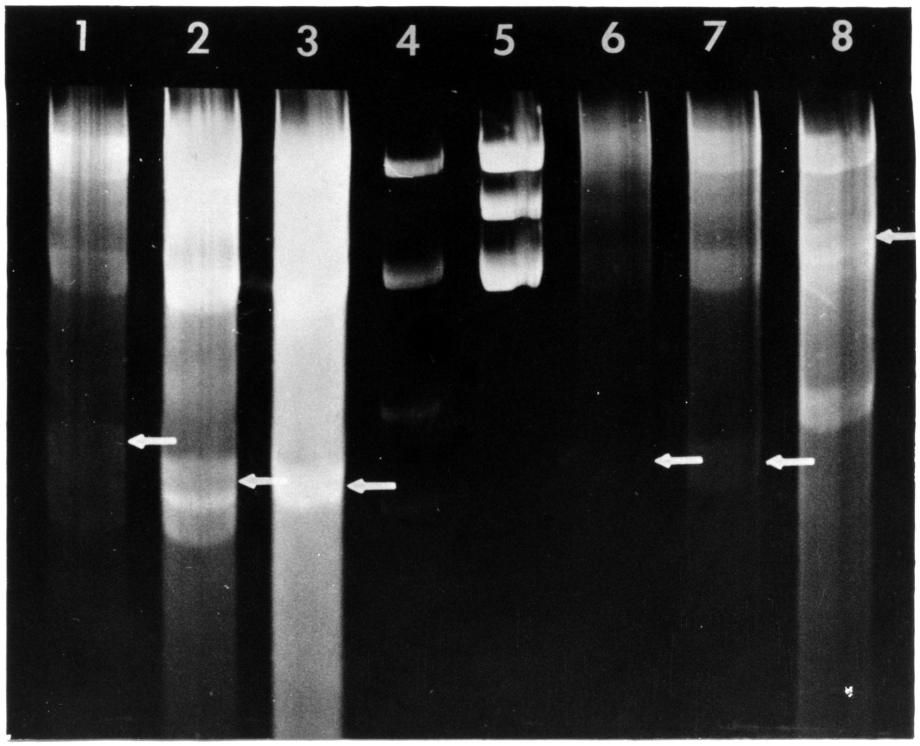


Table 3

INITIAL CHARACTERIZATION

OF DELETION TYPES

DELETION TYPE	FRAGMENTS FUSED	HALO PHENOTYPE
1. EH100	1 & 2	+
2. EN104	1 & 2	-
3. CN106*	1, 2 & 3	-
6. CN105*	1, 2 & 3	-
7. CN104*	1, 2 & 3	-
8. CH100	2, 3 & C	+

* THESE ARE INDIPENDENT ISOLATES FROM A SINGLE LYSATE; ALL THREE APPEAR TO BE IDENTICAL.

Fig. 7 is a represenative gel after the final DNA preparation of the isolates in Table 4.

Figure 7

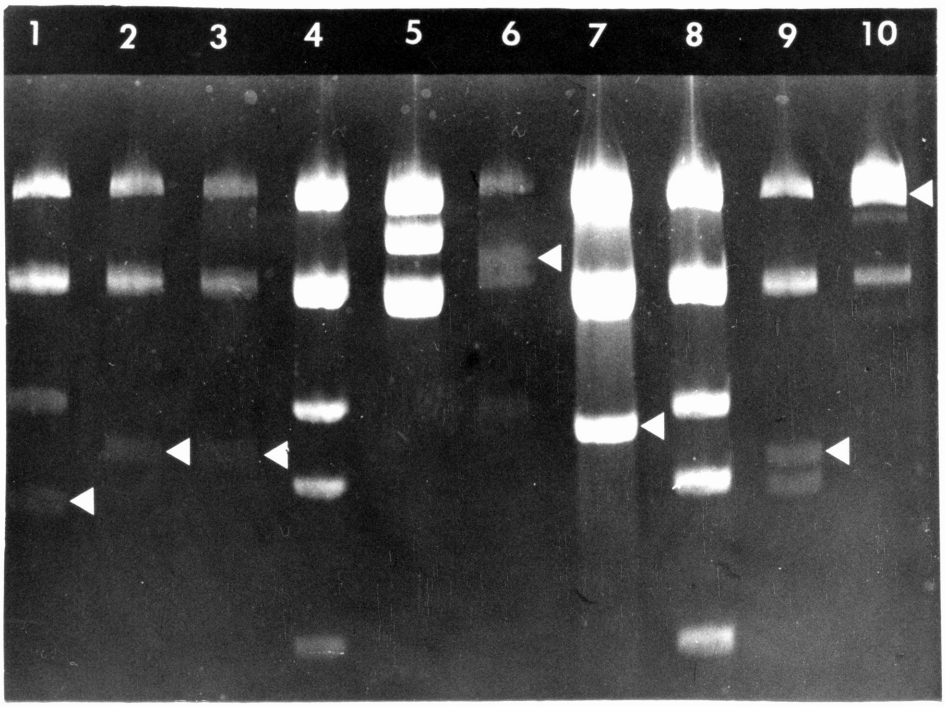


Table 4

MOLECULAR WEIGHTS OF NEW
Sma I RESTRICTION FRAGMENTS
ASSOCIATED WITH DELETION TYPES

DELETION TYPE	FRAGMENTS FUSED	DELETION SIZE (kbp)	MOLECULAR WEIGHT OF NEW FRAGMENT (kbp)
1. DH 101	2 & 3	3.18	4.22
2. CN 105	1, 2 & 3	8.00	4.88
3. CN 104	1, 2 & 3	8.00	4.88
6. CH 100	2, 3 & C	6.50	9.20
7. CN 200	1, 2 & 3	7.65	5.15
9. EH 201	1 & 2	3.60	4.80

Fig. 8 contains the maps obtained from annalysis of electrophoretic gels in Fig. 7.

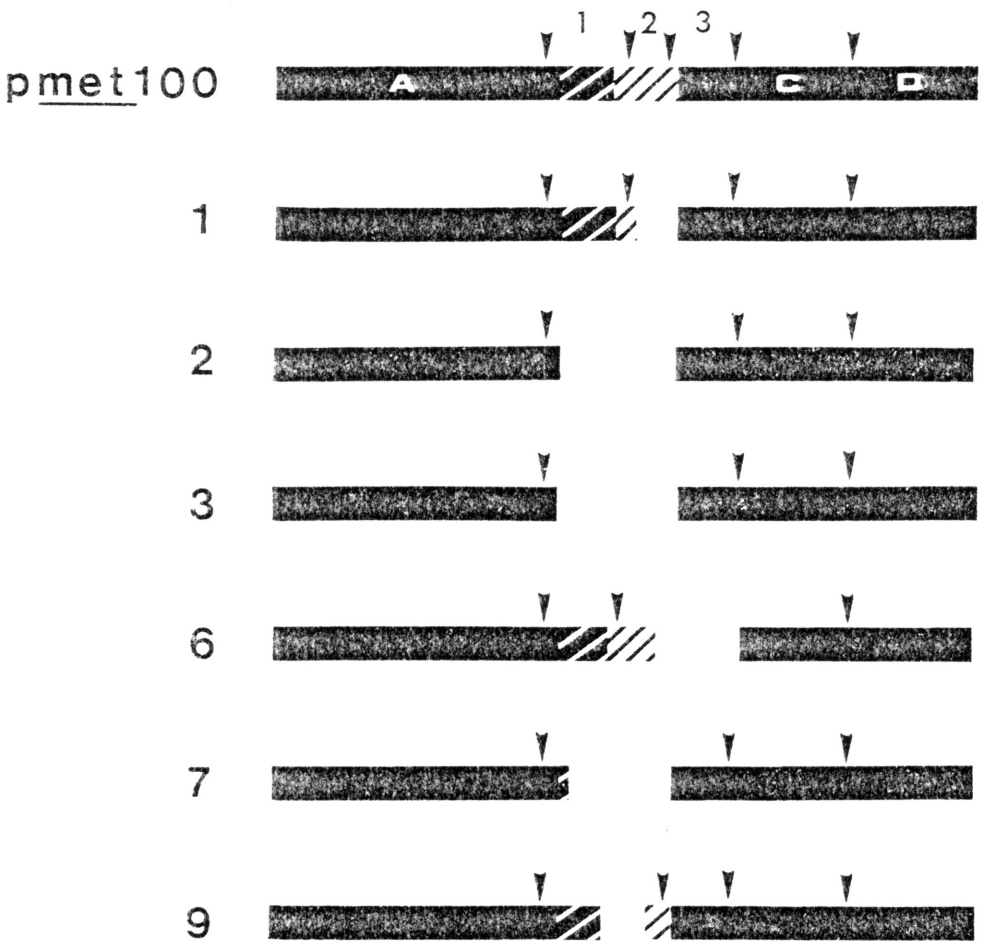
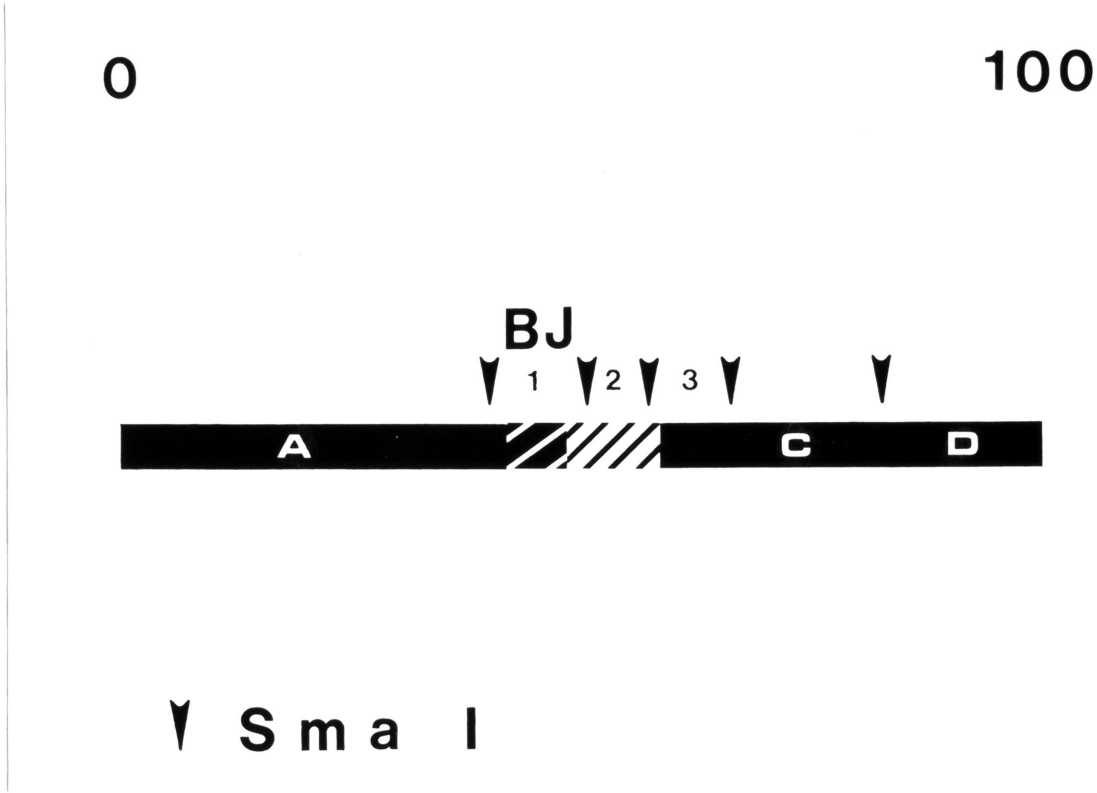

pmet100
1
2
3
A
C
D
1
2
3
6
7
9

Fig. 9 is a detailed map of lambda pmet 100 showing the bands represented in the Sma1 restriction paterns


0
100
BJ
1
2
3
A
C
D
Sma I