

Isolation of Mutants in M13 Coat Protein That Affect Its Synthesis, Processing, and Assembly into Phage*

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The major coat protein (gene 8 protein) of bacteriophage M13 has been studied intensively as a model of membrane assembly, protein packing, and protein-DNA interactions. Because this protein is essential for assembly of the phage, very few mutants have been isolated. We have therefore cloned the gene 8 into a plasmid under control of the *araB* promoter. In the presence of arabinose, the cloned gene is expressed at a rate comparable to that in an M13-infected cell. Plasmid-derived procoat is inserted across the plasma membrane and processed to coat at a normal rate. The coat can support plaque formation by a defective M13 virus (M13am8) with an amber mutation in its procoat gene. This complementation assay was used to screen the mutagenized, cloned gene 8 for mutants which fail to make fully functional coat. Mutants were obtained which fail to synthesize procoat, which do not convert procoat to mature coat protein, or in which the coat protein is incapable of assembling into infectious vi-

M13 and the other filamentous coliphages have received intensive genetic and biochemical characterization during the last 25 years (Denhardt *et al.*, 1978; Webster and Lopez, 1985). Early in these investigations, a large collection of conditional lethal mutants was prepared (Pratt *et al.*, 1966). However, among several hundred mutants in the 10 genes of this virus, none were obtained in gene 8, which encodes the major capsid protein (Henry and Pratt, 1969). Special techniques allowed the selection of one amber mutant in gene 8 (Pratt *et al.*, 1969), and several second-site intragenic pseudorevertants have been obtained and studied (Pratt *et al.*, 1969; Boeke *et al.*, 1980; Zimmermann *et al.*, 1982). This scarcity of gene 8 mutants presumably reflects the multiple interactions of each residue of procoat at different stages of the viral life cycle. These include protein-protein and protein-DNA interactions, tight packing of coat protein in the virion structure, the complex steps of coat protein biosynthesis and virus assembly and, during cell infection, the process of virion disassembly.

M13 coat protein is synthesized as a precursor, termed procoat, with an amino-terminal leader peptide of 23 amino

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acids. This precursor assembles into the inner membrane of *Escherichia coli* and is processed to yield membrane-bound coat protein plus leader peptide (Wickner, 1983). In M13-infected cells, coat protein is an abundant and particularly simple membrane protein of 50 residues. The central 20 residues are entirely apolar and span the membrane. The coat protein assembles onto extruding DNA to form progeny virions. This assembly process is complex, involving viral DNA, several minor capsid proteins, and viral encoded catalysts of assembly as well as the major coat protein itself (Webster and Cashman, 1978). The M13 virus contains no lipid; the apolar protein-lipid interactions of the membrane-bound form of coat protein are replaced by protein-protein interactions in the virus. When the virus infects a cell, the DNA extrudes from its coat as the coat disassembles into the inner membrane. These "parental" coat protein molecules are used dispersively in the assembly of daughter phage (Trenkner *et al.*, 1967; Smilowitz, 1974).

Coat protein has been studied as a model of membrane protein structure (Nozaki *et al.*, 1976; Chamberlain *et al.*, 1978) and of protein insertion into membranes (Wickner, 1983) and as an essential element in virus capsids (Fodor *et al.*, 1981; Thomas *et al.*, 1983). We now report a general technique for readily obtaining mutants in gene 8 which are defective in particular stages of coat protein biosynthesis and the infectious cycle.

EXPERIMENTAL PROCEDURES

Bacteria, Phage, and Plasmids—*E. coli* K12 HJM114 [Δlac pro F' (lac pro)] was from our collection (Wickner and Killick, 1977). *E. coli* K12 WA921 [met leu lac F' (lac)] was used as suppressor strain and has been recently described (Kuhn *et al.*, 1983). Wild-type M13 and M13 with an amber mutation in gene 7 (M13am7 H2) and in gene 8 (M13am8 H1) as well as its pseudorevertant (M13am8 H1R1) were from Dr. David Pratt (University of California at Davis). M13mp9 was purchased from Pharmacia.

The plasmid pING-1 containing the *araB* promoter and the amino-terminal part of the *araB* gene, as well the *ara* regulatory region and the *araC* gene (Johnston *et al.*, 1985), was a gift from Dr. Gary Wilcox (University of California at Los Angeles). The plasmids pQN805, pQN810, pQN868, and pQN873 containing the M13 gene 8 were constructed as described below. M13 replicative form I DNA was prepared as described previously (Hines and Ray, 1980), and plasmid DNA was isolated using the alkaline SDS¹ procedure (Birnboim and Doly, 1979).

DNA Techniques—Restriction enzymes, T4 ligase, and T4 polymerase were purchased from New England Biolabs, bacterial alkaline phosphatase and Klenow fragment were from Boehringer Mannheim, and radioactive nucleotides were from Amersham Corp. Restriction fragments were isolated from agarose gels by adsorption onto NA45 DEAE paper (Schleicher & Schuell). Transformation followed the calcium shock procedure of Cohen *et al.* (1973).

Mutagenesis—Plasmid DNA (10 µg in 10 µl) was treated with 600

¹ The abbreviation used is: SDS, sodium dodecyl sulfate.

mm hydroxylamine (Sigma), 700 mm phosphate buffer (pH 6), and 1 mm EDTA at 70 °C for 3 or 4 h (Völker and Showe, 1980). The hydroxylamine was then removed by filtration through Sephadex G-25.

Protein Labeling Procedures and Polyacrylamide Gel Electrophoresis—Overnight cultures of HJM114, grown in M9 minimal medium containing 0.2% glucose, were diluted 1:100 into M9 medium containing 0.2% arabinose and shaken at 37 °C to midlog phase ($A_{550} = 0.2$). In some experiments, cells were infected with M13 (at $A_{550} = 0.1$) at a multiplicity of 100 and cultured for 60 min. Aliquots (100 μ l) were labeled with 10 μ Ci of [³⁵S]methionine (>1000 Ci/mmol, Amersham Corp.) and chased with an excess of nonradioactive methionine (1 mg/ml). The samples were mixed with an equal volume of chilled 40% trichloroacetic acid. Precipitates were collected by centrifugation, twice resuspended with ice-cold acetone, sedimented, resuspended in 50 μ l of 10 mM Tris-HCl (pH 8) containing 2% SDS, and incubated for 5 min at 95 °C. Aliquots (5 μ l) were mixed with an equal volume of sample buffer and applied to 19% polyacrylamide gels in SDS containing 6 M urea. After electrophoresis, the gels were fixed (Ito *et al.*, 1980), incubated in salicylate, dried, and fluorographed (Chamberlin, 1979).

Immunoprecipitations—Samples were precipitated with acid and acetone, dissolved in SDS as described above, and immunoprecipitated (Zimmermann and Wickner, 1983). To reduce nonspecific binding, formalin-fixed *Staphylococcus aureus* (Kessler, 1975) was mixed with the samples prior to immunoprecipitation and removed by centrifugation. Radioactivity of procoat and coat was quantified from the flurograms by the method of Suissa (1983).

RESULTS

Strategy of the Experiments (Fig. 1)—Since the M13 gene 8 is essential for the life cycle of the M13 virus, only conditionally lethal gene 8 mutants of M13 are viable. In order to obtain a wide variety of mutants, we have cloned gene 8 into a plasmid, which, unlike the M13 virus, will not require functional coat protein for its continued presence in the cell. Placing the procoat gene behind an inducible and repressible promoter assures that the sequence of a mutant gene will

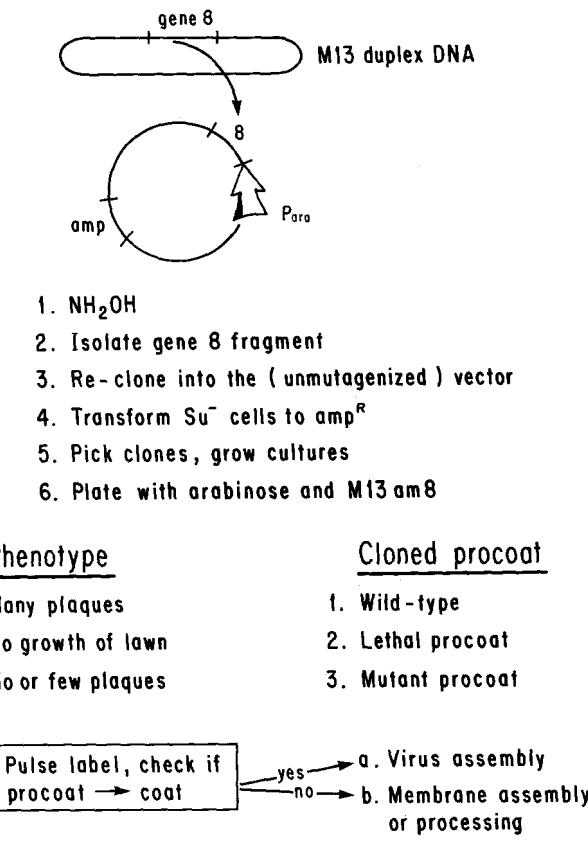


FIG. 1. Strategy for isolating mutants of procoat.

have no effect on cell growth. The cloned gene 8 was mutagenized *in vitro* with hydroxylamine, re-excised from the plasmid, and ligated into a non-mutagenized plasmid. In this fashion, the DNA can be heavily mutagenized and all mutations in the plasmid will be in gene 8. Any mutant plasmid can be transformed into the bacterial cell in the absence of inducer, allowing isolation of lethal gene 8 mutants. Production of coat protein from the plasmid-born wild-type gene was sufficient to allow M13 amber 8 plaque formation. Transformants bearing the plasmid with mutagenized gene 8 were induced with arabinose and tested for production of functional coat protein by plating with M13am8. Mutations in gene 8 which affect any step of the coat protein "life cycle" could be recognized because they prevented plaque production. Mutant procoats were then categorized by investigating their synthesis, processing to coat, and assembly into virus.

Isolation and Cloning of M13 Gene 8—M13 duplex DNA (replicative form I) was digested with the restriction enzymes *Rsa*I and *Ban*I (Fig. 2A, lane 2), yielding a 520-base pair fragment (residues 1249-1769) that contained the gene 8 without its promoter (lane 2, arrowhead). To remove a co-migrating 514-base pair fragment (residues 5486-6000), the DNA was further digested with *Hpa*II, which does not cleave the 520-base pair gene 8 fragment (lane 3). The latter fragment was isolated from an agarose gel by adsorption to DEAE paper (lane 4). The CGTG overhang at the 5' end of this fragment was filled in by incubation with T4 polymerase, dCTP, dGTP, and [α -³²P]dATP (lane 5). The identity of the M13 gene 8 fragment was verified by digestion with the restriction enzyme *Hae*III. As expected, only one fragment, corresponding to the CGTG overhang, was radioactively labeled (lanes 6 and 7).

The blunt-ended gene 8 fragment was cloned into the pING-1 vector (Fig. 2B), a pBR322-derived plasmid which carries the regulatory elements (*araC*) and the promoter of the L-ribulokinase (*araB*) (Johnston *et al.*, 1985). The plasmid DNA was cleaved with *Sma*I and treated with bacterial alkaline phosphatase to prevent self-closure during the subsequent ligation. The M13 gene 8 fragment and the open vector were mixed in a 10:1 molar ratio, joined by DNA ligase, and transformed into *E. coli* HJM114. Ampicillin-resistant colonies were isolated, and the size of their plasmid was determined in minilysates. Restriction analysis with the enzyme *Dde*I (data not shown) revealed whether the gene 8 fragment was inserted into the vector in the sense orientation (plasmids pQN805 and pQN810) or the antisense orientation (pQN868 and pQN873).

Expression of the Cloned Procoat Gene—The *Sma*I site of the pING-1 vector, where the gene 8 was cloned, is in the structural region of the *araB* gene. As predicted from the DNA sequence, neither orientation of the cloned M13 gene 8 was in the same reading frame as *araB*, and therefore the cloning did not produce a fusion protein. As shown in Fig. 3A, pulse-labeled cells with the cloned procoat gene in the sense orientation showed an arabinose-inducible expression of M13 coat (lanes 6 and 8) which was not seen without inducer (lanes 5 and 7), in cells without plasmid (lanes 1 and 2), in cells with the pING-1 plasmid without insert (lanes 3 and 4), or in cells with the gene 8 inserted in the vector in the antisense orientation (lanes 9 and 10). The identities of procoat and coat were confirmed by immunoprecipitating extracts of these pulse-labeled cells with antiserum to coat protein (Fig. 3B). Expression of procoat did not alter the growth rate of the culture (data not shown).

Previous studies of procoat synthesis, membrane assembly, and conversion to coat protein have been carried out in virus-

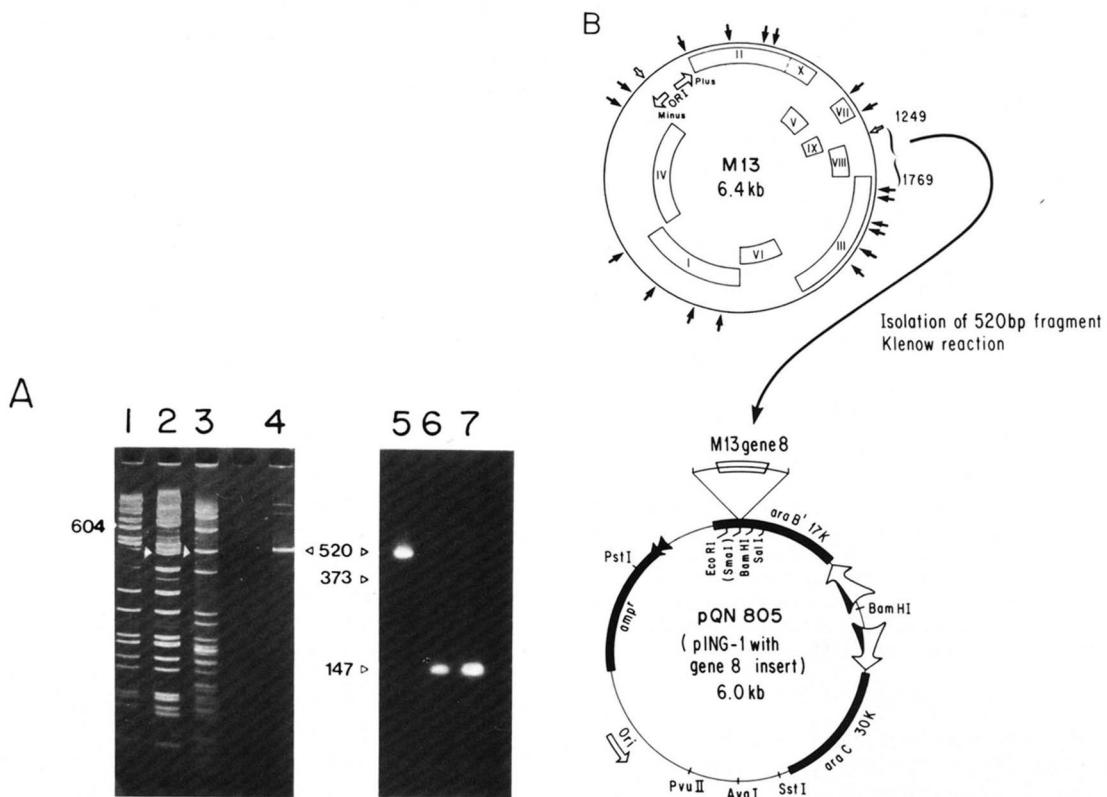


FIG. 2. Isolation and cloning of M13 gene 8. *A*, M13 replicative form I DNA was digested by restriction enzymes *Rsa*I (*lane 1*), *Rsa*I and *Ban*I (*lane 2*), or *Rsa*I, *Ban*I, and *Hpa*II (*lane 3*). The 520-base pair fragment was isolated (*lane 4*), incubated with Klenow enzyme (*lanes 5 and 6*) or with T4 polymerase (*lane 7*) in the presence of dCTP, dGTP, and [α^{32} P]dATP, and then digested with *Hae*III (*lanes 6 and 7*). The DNA was separated on an 8% polyacrylamide gel and stained with ethidium bromide (*lanes 1-4*) or autoradiographed (*lanes 5-7*). *B*, M13 gene 8, isolated by digestion of M13 duplex DNA with the restriction enzymes *Rsa*I (solid arrows) and *Ban*I (open arrows), was cloned into the *Sma*I site of pING-1, a pBR322-derived vector, that carries the *araC* coding and regulating region as well the promoter of *araB* and the amino-terminal *araB* coding region. *bp*, base pair; *kb*, kilobase pair.

infected cells (Date *et al.*, 1980; Date and Wickner, 1981; Zimmermann *et al.*, 1982). To determine whether the phage encodes other proteins which are themselves essential for coat protein biosynthesis, we have compared plasmid- and phage-encoded procoats for their rate of synthesis and kinetics of processing to coat protein (Fig. 4). In cells infected by wild-type M13 (*lane 2*), some of the procoat was already converted to coat after a 15-s pulse of [35 S]methionine. Uninfected cells with the induced pQN805 plasmid showed similar rates of procoat synthesis and conversion to coat protein (*lane 6*). In cells infected by M13am7 virus, either with or without the plasmid pQN805 (*lanes 3 and 8*), there was a higher proportion of the procoat which was not yet converted to coat, in agreement with previous reports (Ito *et al.*, 1979). The proportion of plasmid-encoded procoat which was processed during the 15-s pulse-labeling period was not affected by M13am8 infection (*lane 9*). These results demonstrate that the other viral genes have no necessary role in the synthesis of M13 procoat or in its membrane assembly and conversion to coat protein.

M13-infected cells or uninfected cells expressing the plasmid-borne gene 8 showed a similar rate of conversion of procoat to coat (Fig. 4). However, plasmid-encoded coat protein was degraded in uninfected cells (half-life \approx 4 min) and was completely proteolyzed after 30 min (Fig. 5A). Coat protein is not degraded in M13am7-infected cells (Fig. 5B) where there is no virus assembly (Pratt *et al.*, 1969), suggesting that it is not merely the virus assembly which protects coat from degradation. This suggests that there are factors in virus-

infected cells which prevent coat protein degradation.

Protease accessibility was used to determine the location of the procoat and coat (Fig. 6). Cells bearing the procoat expression plasmid pQN805 were grown in the presence of arabinose and pulse-labeled for 1 min with [35 S]methionine. The outer membrane of the cells was permeabilized by treatment with Tris/sucrose/EDTA at 0 °C (Fig. 6A, *lane 1*), followed by incubation for various times with proteinase K (*lanes 2-5*). The coat protein was accessible to the proteinase added to the outer surface of the plasma membrane, whereas the procoat was not degraded. Protection of procoat was lost when the plasma membrane was disrupted by detergent (*lane 6*). We observed the same digestion patterns when the cells were infected by M13am8 virus (Fig. 6B), again suggesting that the other virus genes have little effect on the location of procoat within the cell. As previously reported (Date *et al.*, 1980), this same orientation pattern is seen in M13 or M13am7-infected cells without pQN805 (Fig. 6C).

An Assay for Functional Plasmid-borne Coat Protein—Since the synthesis of coat protein in cells with plasmid-borne gene 8 is as vigorous as in virus-infected cells (Fig. 4), we tested whether this coat protein could support virus assembly. Cells expressing coat protein from the plasmid-borne gene were infected with M13am8 phage (Fig. 7B). Pulse-labeled coat protein appeared (as virus) in the centrifuged culture supernatant. The chase of coat protein into virus was similar to that seen in cells infected with the wild-type virus (Fig. 7A). In contrast, coat protein expressed from the plasmid was not

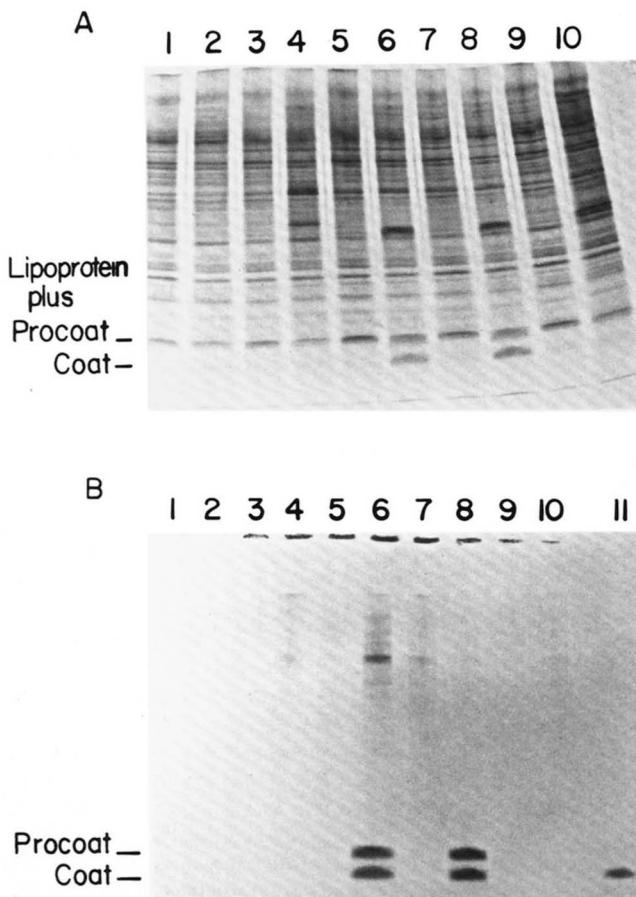


FIG. 3. Arabinose-induced expression of plasmid-derived procoat. Exponentially growing cells in M9 minimal medium containing 0.2% glucose (lanes with odd numbers) or 0.2% arabinose (lanes with even numbers) were labeled with [³⁵S]methionine for 15 s and analyzed by SDS-polyacrylamide gel electrophoresis (A) or immunoprecipitated with antisera to coat protein prior to electrophoresis (B). *E. coli* HJM114 (lanes 1 and 2) and HJM114 containing plasmids pING-1 (lanes 3 and 4), pQN805 (lanes 5 and 6), pQN810 (lanes 7 and 8), or pQN868 (lanes 9 and 10) were analyzed. Lane 11 shows a coat standard from isolated M13 phage, which was labeled with [³⁵S]methionine.

found in the culture supernatant in cells infected by M13am7, a mutation which blocks virion assembly (Fig. 7C), or in uninfected cells (Fig. 7D).

Plaque formation was used as an independent assay that M13am8 virus can assemble using coat protein encoded by the pQN805 plasmid. As shown in Table I, the M13am8 phage stock was contaminated with wild-type revertant phage at a level of 3×10^{-5} . The plating efficiency was increased to 5×10^{-3} on lawns of bacteria with pQN868, where the procoat gene is cloned in the reverse orientation and cannot be expressed. Similarly, in the case of the repressed expression of the procoat gene from plasmid pQN805, the plating efficiency was 3×10^{-3} . This increase in the plating efficiencies was due to recombination between the M13am8 virus and pQN805 and pQN868, as shown by the fact that the virus in these plaques was of wild-type genotype rather than amber. Full plating efficiency was restored by inducing coat protein synthesis with arabinose in cells bearing the gene 8 in the correct orientation. However, these plaques contained considerably fewer phage particles than the plaques obtained from wild-type or suppressed amber phages, suggesting that the rate of virus production was not fully restored to wild-type levels. Moreover, 40% of the virus in these plaques was wild-type,

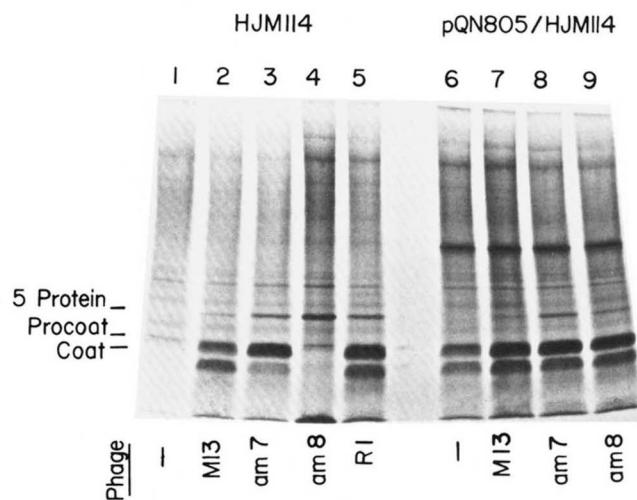


FIG. 4. Synthesis and processing of procoat derived from phage or plasmid. *E. coli* HJM114 (lanes 1–5) or HJM114 with pQN805 (lanes 6–9) was grown in the presence of 0.2% arabinose to a density of 10^8 cells/ml. The cells were infected with M13 (lanes 2 and 7), M13am7 (lanes 3 and 8), M13am8 (lanes 4 and 9), or M13am8 R1 (lane 5) at a multiplicity of 100 and grown for 1 h. Cells were pulse-labeled for 15 s with [³⁵S]methionine and mixed with an equal volume of ice-cold 40% trichloroacetic acid. The precipitates were then analyzed by gel electrophoresis and fluorography as described under "Experimental Procedures."

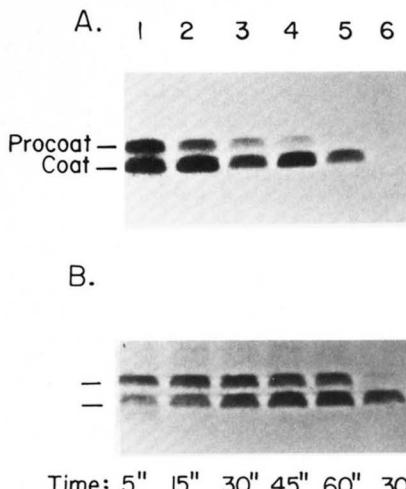


FIG. 5. Plasmid-borne procoat is processed to coat and degraded. Exponentially growing cells in minimal medium containing 0.2% arabinose were pulse-labeled with [³⁵S]methionine for 15 s and chased with excess of L-methionine for the times indicated. A, uninfected HJM114 with pQN805; B, HJM114 infected with M13am7. The samples were precipitated with trichloroacetic acid and processed for immunoprecipitation, gel electrophoresis, and fluorography.

presumably reflecting recombination events in the many rounds of virus growth during plaque formation.

Mutagenesis of the Procoat Gene—The pQN805 plasmid DNA was mutagenized *in vitro* by incubation with 0.6 M hydroxylamine for 3–4 h at 70 °C. After removal of the mutagen, the gene 8 fragment was isolated by digestion with EcoRI and SalI, agarose gel electrophoresis, and elution onto DEAE paper. This gene 8 DNA was ligated with non-mutagenized pING-1 DNA, which had been cleaved by the same two enzymes. By this means, the hydroxylamine mutagenesis was localized to the gene 8; even the promoter region which controls gene 8 expression was replaced by a (non-mutagenized) arabinose-regulated promoter. This technique allows a

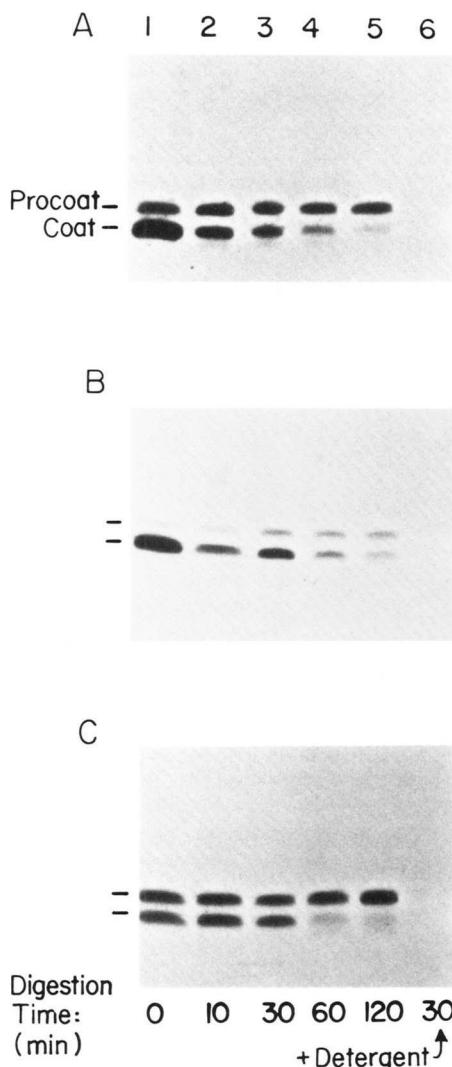


FIG. 6. Plasmid-encoded coat protein spans the plasma membrane of *E. coli*. Uninfected pQN805/HJM114 (A), pQN805/HJM114 infected with M13am8 (B), or HJM114 infected with M13am7 (C) was grown in M9 minimal medium containing 0.2% arabinose. Mid-exponential cultures were pulse-labeled for 1 min with [³⁵S]methionine and osmotically shocked by treatment with ice-cold 60 mM Tris-HCl (pH 8), 40% sucrose, and 20 mM EDTA. Proteinase K was added to a final concentration of 1 mg/ml and incubated at 0 °C for 10 min (lane 2), 30 min (lane 3), 1 h (lane 4), and 2 h (lane 5). A portion of cells were broken by addition of 2% Triton X-100 and incubated for 30 min (lane 6). Proteinase K was inactivated by addition of 1 mg/ml phenylmethylsulfonyl fluoride. Undigested (lane 1) and protease-digested samples were acid-precipitated and processed for immunoprecipitation, gel electrophoresis, and fluorography.

high level of mutagenesis, which is important because of the small size of the procoat gene. The plasmid DNA was then transformed into HJM114, a nonsuppressor strain of *E. coli*, and ampicillin-resistant colonies were selected. One thousand transformants were grown in tryptone broth and plated with M13am8 virus in the presence of arabinose. Forty-two mutants were scored which did not support plaque formation. The mutants were further characterized by testing for interference with M13 wild-type infection. Eight of the 42 mutants were found to be dominant in that they prevented the propagation of both M13am8 and the wild-type phage. All mutants were then further screened for inhibition of plaque formation by wild-type M13 and for their capability to synthesize procoat and process it to coat (Table II). One example of each

class is shown in Fig. 8. The cells containing wild-type (lanes 1 and 2) or mutant plasmids were labeled for 15 s with [³⁵S]methionine (lane 1) and then chased with unlabeled methionine for 60 s (lane 2). The dominant mutant H1 (lanes 3 and 4) and the recessive mutant H7 (lanes 11 and 12) showed normal processing of the procoat to coat. Both classes (class 4 and 5) therefore describe mutants that are affected for the assembly of the phage. The class 1 mutants (such as H3) showed no synthesis of procoat (lanes 5 and 6), and the procoat of H4 (class 2) was rapidly degraded (lanes 7 and 8). The procoat mutants of class 3 (such as H5) were not processed to coat (lanes 9 and 10). Three of the class 3 mutants, which affect a step in membrane assembly, have been investigated in detail and are presented in the accompanying paper (Kuhn and Wickner, 1985).

DISCUSSION

M13 coat protein is an amazingly versatile protein which passes through several distinct environments during its life cycle. In its precursor form (procoat), it is a soluble protein (Ito *et al.*, 1980) which rapidly assembles into the plasma membrane in a transmembrane conformation (Zimmermann *et al.*, 1982; Dalbey and Wickner, 1985). Cleavage of its leader peptide converts it to coat, which spans the plasma membrane (Ohkawa and Webster, 1981). The coat protein leaves the lipid bilayer as it assembles into a regular, crystal-like packing in the virus. Its basic, carboxyl-terminal domain interacts with the viral DNA while its amino terminus is on the outer surface of the phage, interacting with solvent (Marvin and Wachtel, 1975; Nozaki *et al.*, 1976). All of these conformational changes, allow by certain amino acid residues of this small protein, allow basic biological processes, such as insertion of a protein into a membrane, binding of a protein to DNA, and the extrusion of a complex (the phage) across the inner and outer membrane. Mutants in the coat protein will provide important information as to the critical amino acids that are involved in the several distinct reactions of the life cycle of the M13 coat.

We describe a general method for the isolation of mutants in M13 gene 8 encoding procoat. This method combines techniques of cloning and localized mutagenesis with subsequent screening of individual colonies for biological function (e.g., supporting virus assembly). The coding region of the gene spans only 219 base pairs, and potent chemical mutagens must be used to achieve a high frequency of mutations. Hydroxylamine, employed in this study, causes transition mutations. Examination of the gene 8 sequence shows that base transitions could cause a possible 68 mutations in 50 of the 73 amino acid residues of procoat. Other mutagenesis strategies could yield additional mutants. We have screened 1000 colonies with plasmids bearing mutagenized gene 8. Forty-two of these did not support plaque formation by M13am8 virus, and for some of these, we have confirmed by DNA sequencing that they are single-base change transitions (data not shown).

The mutants fall into several broad categories described in Table II. One group of mutants (class 1) showed no detectable expression of radiolabeled procoat. The DNA sequence of one of these showed a base exchange in the starting ATG triplet and two others have been shown to contain an ochre mutation; examination of these nonsense mutants in different suppressor backgrounds (which insert different amino acids) may yield distinct phenotypes. Several mutants (class 3) have been found which make procoat but do not make mature coat protein; these are intriguing from the perspective of membrane biogenesis (Kuhn and Wickner, 1985).

M13 Procoat Mutants

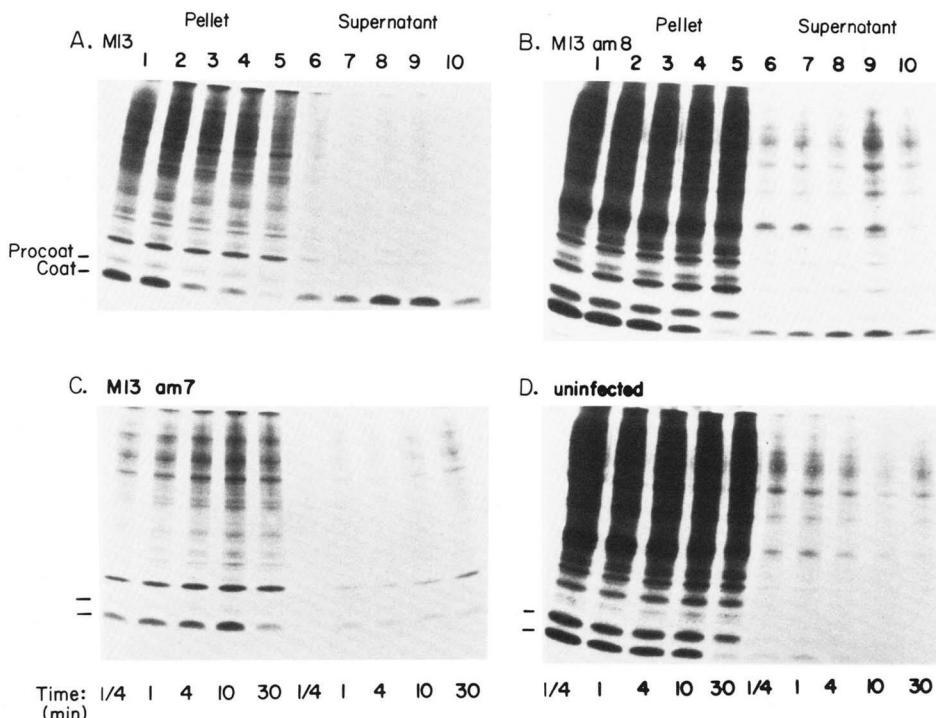


FIG. 7. Plasmid-encoded coat supports M13am8 virus secretion. *E. coli* HJM114 (*A* and *C*) and pQN805/HJM114 (*B* and *D*) growing exponentially in minimal medium containing 0.2% arabinose were infected with wild-type M13 (*A*), M13am8 (*B*), and M13am7 (*C*), or were not infected (*D*). The cultures (1 ml) were pulse-labeled with 50 μ Ci of [35 S]methionine for 1 min and chased with 1 mg/ml L-methionine for 15 s (*lanes 1* and *6*), 1 min (*lanes 2* and *7*), 4 min (*lanes 3* and *8*), 10 min (*lanes 4* and *9*), and 30 min (*lanes 5* and *10*). Then the samples were centrifuged for 10 min in an Eppendorf centrifuge. The resuspended cell pellets (*lanes 1-5*) and the growth medium, supernatants (*lanes 6-10*) were precipitated with 20% trichloroacetic acid and processed for gel electrophoresis and fluorography.

TABLE I
Infections of M13am8 are restored by expression of plasmid-born procoat

Strain	Plating efficiency		Progeny virus (+ara) genotype
	-ara	+ara	
WA921 (sup D)	1	1	Amber
HJM114 (sup+)	0.00003	0.00003	Wild
pQN805/HJM114	0.003	1	Amber/wild
pQN868/HJM114	0.005	0.004	Wild

TABLE II
Phenotype of procoat mutants

The parentheses indicate that class 2 mutant procoats are unstable.

Class	Plaque formation by		Procoat synthesis	Procoat processing	No. of mutants
	M13am8	M13			
1	-	+	-		10
2	-	+	(+)	-	7
3	-	+	+	-	12
4	-	+	+	+	5
5	-	-	+	+	8

Finally, we have obtained mutants which make coat protein yet do not support M13am8 plaque formation; the virus assembly, stability, and (upon infection) disassembly of these mutants may provide information about these processes. For the assembly of progeny virus, the membrane-bound coat protein has to interact with the gene products 7 and 9, which are structural components of virions, and a specific sequence of the single-stranded DNA, the so-called "morphogenetic signal" (Dotto *et al.*, 1981). It is thought that a set of five coat molecules bind to the initiation complex in a cooperative manner (Marzec and Day, 1983), as the phage particle has a barrel-like arrangement of five helices around the DNA. The coat protein molecules in the phage particles have their COOH termini on the inside of the sheath structure (Webster and Cashman, 1978). Their amino-terminal region overlaps the

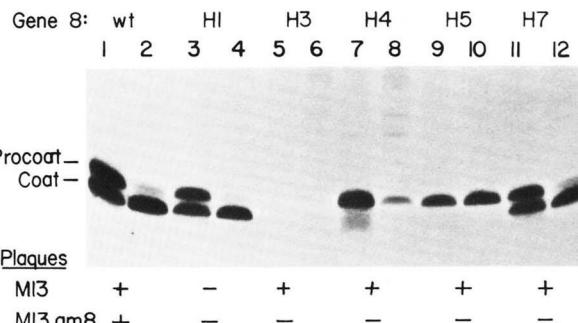


FIG. 8. Various phenotypes of procoat mutants. Cells with the wild-type plasmid pQN805 (*lanes 1* and *2*) and the mutants H1 (*lanes 3* and *4*), H3 (*lanes 5* and *6*), H4 (*lanes 7* and *8*), H5 (*lanes 9* and *10*), and H7 (*lanes 11* and *12*) were pulse-labeled with [35 S] methionine for 15 s (*lanes with odd numbers*) and chased with 1 mg/ml L-methionine for 60 s (*lanes with even numbers*). The samples were then precipitated with 20% trichloroacetic acid and processed for immunoprecipitation, gel electrophoresis, and fluorography.

next set of five coat proteins, forming a second outer layer in a shingle or bottle-brush fashion. It has been proposed that the positively charged amino acids at the COOH terminus of coat interact with the phosphodiester backbone of the DNA (Marvin and Wachtel, 1975) and drive the growing phage out of the cell. Sequence analysis of the mutants which fail to interact with the DNA or with the other capsid subunits will provide a critical test of our concepts of phage secretion and structure. Some mutants have a negative, dominant effect regarding the plating efficiency of wild-type phage. The dominant mutant coat may interact with components of the wild-type virus, preventing normal secretion or stability of progeny phage.

By cloning gene 8, it has now become possible to study the kinetics of expression and processing of procoat without the other phage genes involved. The plasmid-encoded, wild-type procoat is converted to coat as fast as in virus-infected cells. This supports the notion that this reaction only employs

components of the normal host cell. Plasmid pQN805-synthesized coat is degraded with a half-life of approximately 4 min. This is not the case in virus-infected cells, even when virion assembly is blocked (as by the amber 7 mutation). This may provide clues to the mechanisms of protein turnover and how the virus protects coat protein prior to phage extrusion.

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