Fine Structure Genetic and Physical Map of the Gene *3* to *10* Region of the Bacteriophage P22 Chromosome

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ABSTRACT

The mechanism by which dsDNA is packaged by viruses is not yet understood in any system. Bacteriophage P22 has been a productive system in which to study the molecular genetics of virus particle assembly and DNA packaging. Only five phage encoded proteins, the products of genes *3, 2, 1, 8* and *5*, are required for packaging the virus chromosome inside the coat protein shell. We report here the construction of a detailed genetic and physical map of these genes, the neighboring gene *4* and a portion of gene *10*, in which 289 conditional lethal *amber, opal, temperature sensitive* and *cold sensitive* mutations are mapped into 44 small (several hundred base pair) intervals of known sequence. Knowledge of missense mutant phenotypes and information on the location of these mutations allows us to begin the assignment of partial protein functions to portions of these genes. The map and mapping strains will be of use in the further genetic dissection of the P22 DNA packaging and prohead assembly processes.

THE life cycle of the *Salmonella typhimurium* lysogenic bacteriophage P22 has been the subject of extensive genetic and biochemical investigations that have made it one of the most well understood viruses (SUSSKIND and BOTSTEIN 1978; POTEETE 1988). One of the interesting but incompletely understood aspects of P22 growth is the packaging of the dsDNA chromosome within the coat protein shell. During virion assembly the 43,400-bp phage chromosome is packaged by a complex series of reactions in which (1) phage concatemeric DNA is recognized as the proper substrate for packaging, (2) DNA enters precursor particles called proheads, (3) chromosome length DNA molecules are nucleolytically cleaved from precursor DNA, (4) a major precursor particle protein (scaffolding protein) leaves the structure and remains intact to reassemble into new proheads, (5) the coat protein shell expands about 11% in radius, (6) ATP is cleaved, and (7) the products of genes *2* and *3* act but are not found in the completed virion (reviewed by CASJENS 1989). The gene *3* protein forms a complex with gp*2*[3] (POTEETE and BOTSTEIN 1979), and it is at least partially responsible for recognition of phage DNA (RAJ, RAJ and SCHMIEGER 1974; JACKSON, LASKI and ANDRES 1982; CASJENS *et al.* 1987). Both the gene *2* and *3* proteins are required for cleavage of the DNA concatemer (LASKI and JACKSON 1982). DNA insertion into the prohead is thought to begin at a site called *pac* and proceed unidirectionally from that point until the prohead is filled with DNA (103.8% of the sequence), at which point a "headful" cleavage is made in the DNA, freeing the packaged DNA from the concatemer. Subsequent packaging events start from the concatemer end created by the previous event, resulting in processive packaging series typically 2.5 to 5 events long (TYE, CHAN and BOTSTEIN 1974; JACKSON, JACKSON and DEANS 1978; WEAVER and LEVINE 1978; KUFER, BACKHAUS and SCHMIEGER 1982; CASJENS and HUANG 1982; ADAMS, HAYDEN and CASJENS 1983; BACKHAUS 1985; CASJENS and HAYDEN 1988). This is a common replication/packaging strategy for bacteriophages, and in addition, the iridoviruses of animals appear to use a similar strategy (reviewed by CASJENS 1989).

The genes, *3, 2, 1, 8* and *5*, that encode the five P22 proteins required for DNA packaging, lie in a contiguous cluster in the late operon (BOTSTEIN, CHAN and WADDELL 1972; EPPLER *et al.* 1991). The transient function of the gene *3* and *2* proteins in DNA packaging was mentioned above. The gene *1* protein is thought to function as a "portal" through which DNA enters the prohead (BAZINET *et al.* 1988). Scaffolding protein is encoded by gene *8* and forms the internal core of proheads, which leaves the structure during DNA packaging (KING and CASJENS 1974). Coat protein, encoded by gene *5*, forms the outside shell of proheads and completed virions (KING, LENK and BOTSTEIN 1973; EARNSHAW, CASJENS and HARRISON 1976). In order to further our understanding of the genes and proteins that participate in the DNA packaging process, we report here the construction of a detailed genetic and physical map of the region of the bacteriophage P22 chromosome that contains these genes as well as a nonessential

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[3] Abbreviations used: gp*X*, the *g*ene *p*roduct of cistron *X*.

open reading frame ORF109, gene *4* and a portion of gene *10* (EPPLER *et al.* 1991). The products of the latter two genes stabilize the DNA within the head after it is inserted into the coat protein shell (STRAUSS and KING 1984).

A low resolution genetic/physical map of the gene *3* to *10* region of the P22 chromosome has been previously constructed (GOUGH and LEVINE 1968; CHAN and BOTSTEIN 1972; BOTSTEIN, CHAN and WADDELL 1972; RUTILA and JACKSON 1981; WYCKOFF and CASJENS 1985; RIGGS and BOTSTEIN 1987), and the proteins encoded by each of the genes have been identified by SDS-polyacrylamide gel electrophoresis (BOTSTEIN, WADDELL and KING 1973; KING and CASJENS 1974; POTEETE and KING 1977; YOUDERIAN and SUSSKIND 1980). We have recently completed the nucleotide sequence and determined the precise gene placement in this region of the P22 chromosome (EPPLER *et al.* 1991). A large number of conditional lethal mutations have been previously isolated for P22. We present here the use of these mutations and the nucleotide sequence information to construct a very detailed genetic/physical map of this region. One eventual goal is the correlation of partial functions of the proteins involved in DNA packaging with particular portions or domains of the proteins.

MATERIALS AND METHODS

Bacteria, phage and plasmids: *Salmonella typhimurium* DB7000 (*sup°*, *leu am*414) (SUSSKIND, WRIGHT and BOTSTEIN 1974) was used as host for *amber*$^+$ P22 growth. The closely related *amber* suppressing strains DB7154 *sup*D10(Ser), DB7155 *sup*E20(Gln), DB7156 *sup*F20(Tyr) and DB7157 *sup*J60(Leu) (WINSTON, BOTSTEIN, and MILLER 1979) were used for growth of P22 *amber* mutants and to test the suppression patterns of the *amber* mutants. DB109 was used to propagate *ug* phage mutants (CHAN and BOTSTEIN 1972). All Salmonella strains were from the collection of D. Botstein. *Escherichia coli* strain MC1061 (CASADABAN, CHOU and COHEN 1980; RALEIGH *et al.* 1988) was used to carry plasmids. M13 phage vectors and their host are described by YANISCH-PERRON, VIERA and MESSING (1985).

The isolation and description of the P22 conditional lethal mutations used in the construction of the genetic/physical map are described in the references that follow. Allele names beginning with H, N or U were isolated by hydroxylamine, nitrosoguanidine, or UV mutagenesis, respectively (unless otherwise indicated, the mutants were from the collection of D. BOTSTEIN and were gifts from D. BOTSTEIN and A. POTEETE): *ug*H1-*ug*H99 and *am*H200-*am*H299 (LEW and ROTH 1970; *ug* mutations are suppressed by UGA or *opal* suppressors and *am* mutations are suppressed by UAG or *amber* suppressors); *am*H1-*am*H100 (KOLSTAD and PRELL 1969; gifts from H. PRELL and D. BOTSTEIN); *am*N1-*am*N100 and *am*H300-*am*H400 (BOTSTEIN, CHAN and WADDELL 1972); *am*N100-*am*N199 (isolated in the M. LEVINE laboratory–see BOTSTEIN, CHAN and WADDELL 1972); *am*U200-*am*U243 (gift from J. KING and P. PREVELIGE, unpublished; SMITH, BERGET and KING 1980); *am*H1000-*am*H1399 (POTEETE and KING 1977; RIGGS and BOTSTEIN 1987); *am*H1400-*am*H1499 (gift from M. SUSSKIND, unpublished); *ts*1.1-*ts*26.1 (GOUGH and LEVINE 1968); *ts*N1-*ts*N99 (isolated in the M. LEVINE laboratory–see BOTSTEIN, CHAN and WADDELL 1972); *ts* su(*am*UT34)5, *ts* su(*am*UT71)1, *ts* su(*am*Y232)11 and *ts*U172, (gifts from J. KING and P. PREVELIGE, unpublished; BAZINET and KING 1988); *ts*N100-N199, *cs*H1-H199 and other *ts* and *cs* mutations (JARVIK and BOTSTEIN 1973 and 1975; JARVIK 1975; J. JARVICK and D. BOTSTEIN, unpublished); the phage L *amber* mutations were isolated by J. SOSKA (gifts of J. SOSKA and W. BODE; KARLOVSKY *et al.* 1984).

The plasmids constructed for use in the creation of the genetic/physical map are described in Table 1, and their P22 DNA inserts are shown schematically in Figure 1. The locations of the various restriction sites can be found in CASJENS *et al.* (1983), EPPLER *et al.* (1991) and the references therein. Deletion mapping strains f223 through f236 are described by RIGGS and BOTSTEIN (1987).

DNA manipulations: DNA isolation, cleavage by nucleases, end blunting, ligation and transformation were performed as previously described (WYCKOFF and CASJENS 1985; WYCKOFF *et al.* 1986), except that plasmids were moved into Salmonella by electroporation with a Bio-Rad Gene Pulser (25 μF, 1.25 kV, 800 Ω, with 0.2-cm cuvettes containing 1 μl of DNA solution and 40 μl of cell suspension [1×10^{11} cells/ml in 3 mM KPO_4, pH 7.4, 272 mM sucrose, 15% glycerol]). Typically a few hundred transformants of DB7000 were obtained per μg of plasmid DNA from an *E. coli* mini-lysate.

RESULTS

The existing genetic and physical maps of the P22 gene *3* to *10* region were not detailed enough to allow rapid mapping of mutations to particular regions within the genes or to small, easily sequencable regions. We therefore constructed a battery of plasmids containing fragments of P22 DNA from the gene *3* to *10* region and used marker rescue methodology to obtain much more precise locations for a large number of UAG nonsense (*am*), UGA nonsense (*ug* or *opal*), temperature-sensitive (*ts*) and cold-sensitive (*cs*) gene *3* to *10* mutations. For deletion mapping purposes, over forty pBR322 based plasmids were constructed that contain various fragments of P22 DNA from the sequenced region between the start of gene *3* and *Pst*I#3 within gene *10*. They are described in Table 1 and Figure 1. All but one (pUS204H) of these plasmids have P22 DNA endpoints of precisely known sequence. The plasmids were moved into *sup°* *S. typhimurium* DB7000 for marker rescue analysis by electroporation. In this study, we also used for marker rescue ten Salmonella strains containing different P22 phage lysogens which contain deletion endpoints in the region of interest (RIGGS and BOTSTEIN 1987). These endpoints have not been sequenced. Eight of the strains in this set have endpoints which at the current resolution are genetically inseparable from the endpoints defined by our plasmid set. These eight are listed in Table 2 and are not shown in Figure 1. Two deletion lysogens, strains f224 and f228, whose endpoints are genetically separable from the endpoints of our plasmids, are also shown in Figure 1. In addition, the endpoint locations we deduce (from their data) for the deletion lysogen strains used by CHAN

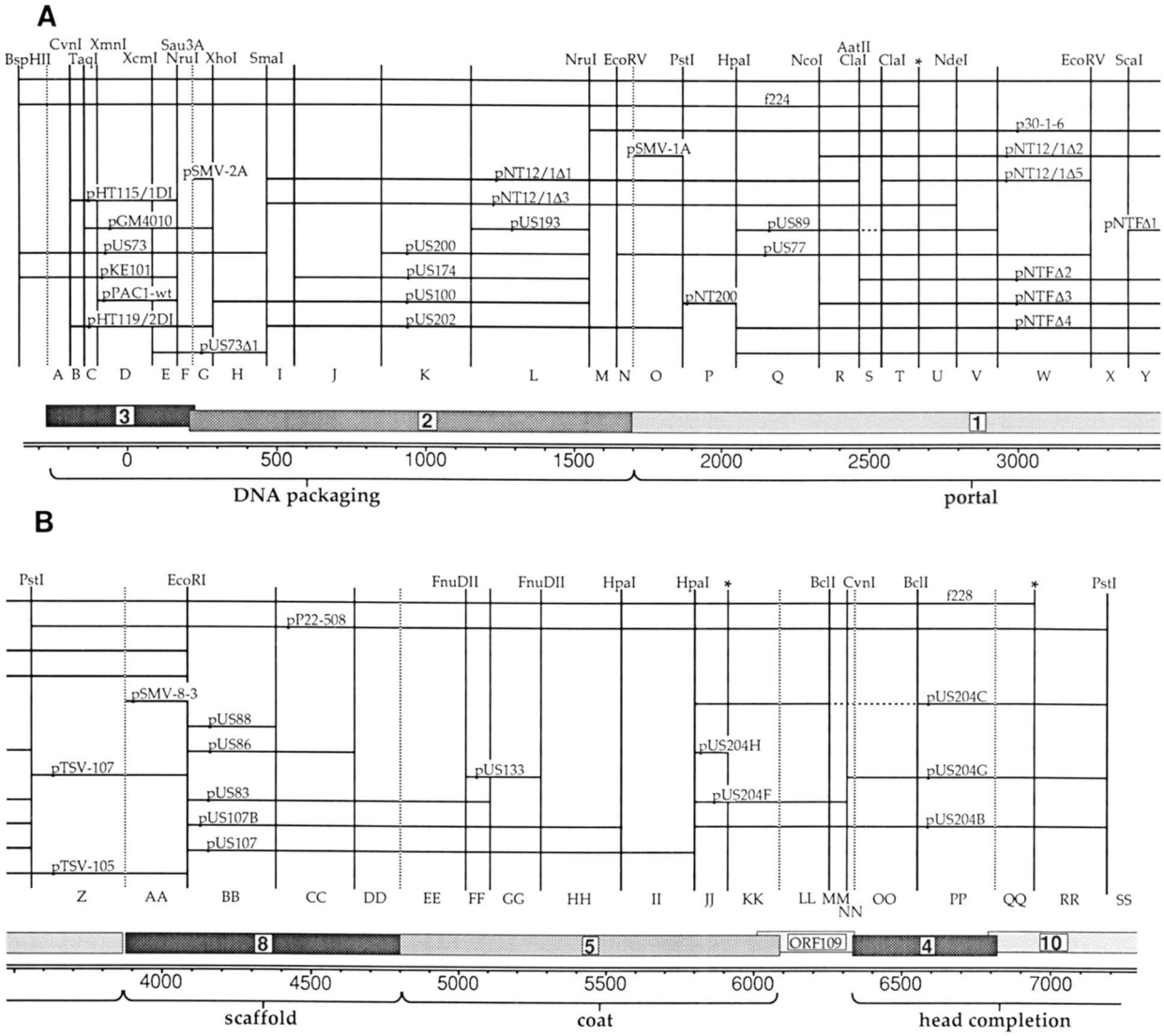

FIGURE 1.——Fine structure genetic and physical map of the phage P22 chromosome region that contains genes *3* to *10*. The stippled bars show the positions of the indicated genes (ORF109 is a nonessential reading frame–EPPLER *et al.* 1991) above a scale in base pairs with the zero point as defined by BACKHAUS (1985). Above, the horizontal solid lines represent P22 DNA fragments cloned into plasmid vectors (see Table 1 and MATERIALS AND METHODS), and dashed horizontal lines indicate deletions within those inserted fragments. The vertical solid lines are the endpoints defined by those DNA fragments, and where relevant the restriction site used to generate the fragment is indicated. If no site is indicated, that end of the P22 DNA insert was generated by exonuclease III-mung bean nuclease deletion (Table 1) or Bal31 digestion (RIGGS and BOTSTEIN 1987). The vertical dotted lines indicate gene boundaries. An asterisk indicates that the endpoint location is imprecisely known (see text). The map intervals are indicated by the letters A through SS (Table 3).

and BOTSTEIN (1972) are also shown in Table 2. The ends of the P22 DNA inserts in the plasmids constructed for this study, along with f224 and f228, define 42 deletion endpoint positions in the region, 39 of which are known to the exact nucleotide. Together with the gene boundaries defined by complementation tests, they create 44 intervals within genes *3* to *10* into which mutant alleles can be mapped.

P22 mutant alleles were located by marker rescue from this set of plasmids in the nonsuppressing host strain *S. typhimurium* DB7000 in manner similar to that described by CHISHOLM *et al.* (1980), except that the analysis was performed as spot tests. Bacterial strains containing plasmids or deletion lysogens were grown overnight at 37°C in L Broth (CHAN and BOTSTEIN 1972) with selecting drug, and marker rescue experiments were performed as spot tests on L plates without drug. The top agar was seeded with 0.2 ml of an overnight culture of a tester host strain (DB7000 carrying one of the above plasmids), 5–10 μl spots containing about 10^2, 10^4 or 10^6 phage (from a single plaque resuspended in 0.066 M $NaPO_4$, pH 7.0, 0.85% NaCl) were placed on the plate and air dried at room temperature (except for *ts* mutations, which were spotted on prewarmed plates on a hot plate at 42°C and air dried at 42°). The plates were then incubated for 18 hr at 37° for UAG nonsense (*am*) and UGA nonsense (*ug*) mutants, 18 hr at 42° for *ts* mutants, and 40 hr at 16.5° for *cs* mutants. In order to consider that a mutation could be rescued by

TABLE 1

Plasmids used in these studies

Strain	Origin
pBR322	Described by BOLIVAR *et al.* (1977)
pGM4010	P22 *Taq*I fragment containing the C-terminal portion of gene *3* inserted into the *Acc*I site of pUC19 (YANISCH-PERRON, VIEIRA and MESSING 1985) in the same translational orientation as the *lacZ* gene fragment (gift of H. SCHMIEGER)
pHT119/2DI	P22 *Cvn*I#6 (filled in)-*Xho*I#1 from P22 HT119/2 inserted between pBR322 *Eco*RI (filled in) and *Sal*I sites
pHT115/1DI	P22 *Cvn*I#6 (filled in)-*Nru*I#1 from P22 HT115/1 inserted between pBR322 *Eco*RI (filled in) and *Eco*RV sites
pKE101	Described by Casjens *et al.* (1989)
pNTF	P22 *Pst*I#1-*Pst*I#2 (from P22 NT5/1) inserted into pBR322 *Pst*I site so that *Pst*I#2 is closest to the vector *Eco*RI site
pNTFΔ1	Deletion of nonorigin containing *Sca*I fragment of pNTF
pNTFΔ2	P22 *Aat*II#1-*Pst*I#2 (from P22 NT5/1) inserted between pBR322 *Aat*II and *Pst*I sites
pNTFΔ3	P22 *Nco*I#1-*Pst*I#2 (from P22 NT5/1) inserted between pBR322 *Ssp*I and *Pst*I sites
pNTFΔ4	P22 *Hpa*I#1-*Pst*I#2 (from P22 NT5/1) inserted between pBR322 *Ssp*I and *Pst*I sites
pNT12/1	P22 *Sma*I#1-*Eco*RI#1 (from P22 NT12/1) inserted between the *Ssp*I and *Eco*RI sites of pBR322
pNT12/1Δ1	*Cla*I fragments without origin deleted from pNT12/1
pNT12/1Δ2	*Nco*I-*Pvu*I (made blunt) fragment without origin deleted from pNT12/1
pNT12/1Δ3	*Pvu*II site in vector-*Nde*I#1 (filled in) fragment without origin deleted from pNT12/1
pNT12/1Δ5	*Eco*RV#2-*Cla*I#2 fragment inserted between the *Ssp*I and *Cla*I sites of pBR322
pNT200	P22 *Pst*I#1-*Hpa*I#1 (from P22 NT5/1) inserted between pBR322 *Pst*I and *Ssp*I sites
pPAC1-wt	P22 DNA from *Xmn*I#1 to *Sau*3A#1 inserted into the modified *Mlu*I site of pP22-10 (RUTILA and JACKSON, 1981)
pP22-508	Described by RUTILA and JACKSON (1981)
pSMV-1A	Polymerase chain reaction (PCR) synthesized P22 DNA from the start codon of gene *1* to *Pst*I#1 inserted into a derivative of pTSV-23 (WYCKOFF *et al.*, 1986–to be described elsewhere)
pSMV-2A	PCR synthesized P22 DNA from the start codon of gene *2* to *Xho*I#1 inserted into a derivative of pTSV-23 as with pSMV-1A
pSMV-8-3	PCR synthesized P22 DNA from the start codon of gene *8* to *Eco*RI#1 inserted into a derivative of pTSV-23 as with pSMV-1A
pTSV-105	P22 *Hpa*I#1 (ligated to *Eco*RI linker)-*Eco*RI#1 inserted into *Eco*RI cut pTSV-3b (WYCKOFF *et al.*, 1986)
pTSV-107	The Lac promotor carrying *Hin*dIII-*Eco*RI fragment from pOP854B (WYCKOFF and CASJENS 1985) was inserted into *Hin*dIII-*Eco*RI cut pTSV-3b (WYCKOFF *et al.*, 1986). The resulting plasmid carries the *Pst*I#2-*Eco*RI#1 region of P22 DNA
pUS73	P22 *Hin*dIII#14-*Sma*I#1 fragment (from P22 HT12/4) inserted between pBR322 *Hin*dIII and *Eco*RI sites[a]
pUS73Δ1	*Xcm*I#1 (made blunt)-*Hin*dIII (filled in) fragment without origin deleted from pUS73
pUS77	P22 *Eco*RV#1-*Eco*RV#2 fragment inserted into the pBR322 *Eco*RI site with *Eco*RI linkers
pUS83	P22 *Eco*RI#1-nucleotide 5105[b] fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites[a]

homologous recombination from a plasmid carrying a P22 DNA insert, we required that its plating efficiency be consistently more than 10-fold greater on a nonpermissive host strain carrying a plasmid with a "rescuing" P22 DNA fragment than on a similar plasmid carrying a non-overlapping P22 fragment. The plating efficiencies of *amber* mutants on hosts carrying "rescuing plasmids" varied from 10^{-1} to about 10^{-4}, with the lowest efficiencies occurring on plasmids with the smallest P22 DNA inserts and with mutations that map very near the end of the P22 DNA insert. For example, *8*$^-$ *ambers* H1348 and H202, which have been sequenced by EPPLER *et al.* (1991), and lie at positions 4127 and 4352, respectively, 50 and 12 bp from the ends of the 290-bp P22 DNA insert in pUS88, had plating efficiencies on hosts carrying this plasmid of 2.5×10^{-4} and 1.0×10^{-4}, respectively. These figures increase substantially when the mutation is not so near the plasmid-P22 DNA junction, as is demonstrated by the plating efficiencies of 4.5×10^{-4} and 2.2×10^{-3} for these same two mutations on a host carrying the plasmid pUS83 (see Figure 1), in which the distance from *am*H202 to the right end is increased to 754 bp, but the left end nearest *am*H1348 is the same as in pUS88. The frequency of *amber*$^+$ revertants in single resuspended plaques was usually between 10^7- and 10^5-fold less than the total number of phage particles; for example, the frequencies of revertants in the *am*H1348 and *am*H202 plaques used above were 1.1×10^{-5} and 3.3×10^{-7}, respectively.

In this way, the locations of 289 conditional lethal mutations, including 62 gene *3*, 48 gene *2*, 63 gene *1*, 28 gene *8*, 71 gene *5*, 2 gene *4*, and 5 gene *10* mutations, were determined. Four of these mutations were isolated in phage L, a P22 relative that has more than 90% DNA sequence identity to P22 in the region under study (HAYDEN, ADAMS and CASJENS 1985). Although the rescue frequencies for these were more than 10-fold lower than those of nearby P22 alleles, unambiguous map positions were obtained. Clearly the mapping strains are also useful for locating phage L conditional lethal mutations. D. BOTSTEIN, J. KING,

Strain	Origin
pUS86	P22 *Eco*RI#1-nucleotide 4647[b] fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites[a]
pUS88	P22 *Eco*RI#1-nucleotide 4384[b] fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites[a]
pUS89	P22 *Hpa*I#1-nucleotide 2927[b] fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites.[a] In addition it carries a spontaneous deletion of the nucleotides between positions 2466 and 2538 as determined by nucleotide sequencing
pUS100	P22 *Xho*I#1-*Nru*I#2 fragment inserted between pBR322 *Hin*dIII and *Eco*RI sites[a]
pUS107	P22 *Eco*RI#1-*Hpa*I#3 fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites[a]
pUS107B	P22 *Eco*RI#1-*Hpa*I#2 fragment inserted between pBR322 *Eco*RI and *Eco*RV sites
pUS133	P22 *Fnu*DII (nucleotide 5029)-*Fnu*DII (nucleotide 5274) fragment inserted between pBR322 *Sal*I and *Eco*RI sites[a]
pUS174	P22 nucleotide 555[b]-*Nru*I#2 fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites[a]
pUS193	P22 nucleotide 1149[b]-*Nru*I#2 fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites[a]
pUS200	P22 nucleotide 849[b]-*Nru*I#2 fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites[a]
pUS202	*Sma*I#1-*Pst*I#1 fragment inserted between pBR322 *Eco*RI and *Hin*dIII sites[c]
pUS204B	P22 *Hpa*I#3-*Pst*I#3 fragment inserted between pBR322[c] *Eco*RI and *Hin*dIII sites[a] (EPPLER *et al.*, 1991)
pUS204C	*Bcl*I deletion of pUS204B
pUS204F	P22 *Hpa*I#3-*Cvn*I#2 (filled in) fragment inserted between pBR322[c] *Eco*RI and *Hin*dIII (filled in) sites[a]
pUS204G	P22 *Cvn*I#2 (filled in)-*Pst*I#3 fragment inserted between pBR322[c] *Eco*RI (filled in) and *Hin*dIII sites[a]
pUS204H	P22 *Hpa*I#3-about nucleotide 5900 (created by an inadvertant deletion in pUS204B) fragment inserted between pBR322[c] *Eco*RI and *Hin*dIII sites[a]
p30-1-6	P22 *Nru*I#2-*Eco*RI#1 fragment (from P22 NT5/1) inserted between pBR322 *Nru*I and *Eco*RI sites

DNA inserted into vectors was from P22 wild-type or *c1-7 13⁻am*H101 phage unless otherwise indicated. The P22 HT and NT DNAs are from phages that contain nonlethal mutations that affect generalized transduction, but do not affect the marker rescue analysis (RAJ, RAJ and SCHMIEGER 1974; our unpublished results). In each plasmid the orientation of the insert can be deduced by the reader from the order in which the P22 cleavage sites and vector insertion sites are given in the table–the first cleavage site given was joined to the first insertion site given, etc. Positions of P22 restriction sites can be found in CASJENS *et al.* (1983), EPPLER *et al.* (1991) and references therein.

[a] P22 DNA insert was first cloned into M13 mp10, mp11, mp18 or mp19 (EPPLER *et al.* 1991) and was subsequently moved into pBR322 after cleavage of the M13 polylinker sites.

[b] This end was generated by unidirectional exonuclease III-mung bean nuclease deletion (HENIKOFF 1984) of a P22 DNA fragment cloned into an M13 vector. The exact nucleotide number of the endpoint was determined by nucleotide sequencing of the resulting M13 phage DNA (see EPPLER *et al.* 1991). The DNA fragment was then moved to pBR322 using compatible restriction endonuclease cleaved ends and the M13 polylinker sites.

[c] pBR322 sequences between *Bam*HI and *Pvu*II were deleted.

M. LEVINE, H. PRELL and M. SUSSKIND were kind enough to give us complete access to their phage P22 strain collections. Thus, we attempted to map all of the currently available conditional lethal mutations in these collections which had previously been located in the gene *3* to *10* region. Only a few of the mutations previously reported in the literature could not be mapped by the techniques used here. Most of these were *ts* and *cs* mutations, for example *3⁻ ts*RH203, *8⁻ cs*RN26, *5⁻ ts*rN26K and *5⁻ ts*rH58A (JARVICK and BOTSTEIN 1975), whose non-permissive phenotypes were too weak to be useful. However, a few *amber* mutations that were previously placed in the gene *3* to *10* region, such as *1⁻ am*N21 (CHAN and BOTSTEIN 1972) and *5⁻ am*H1133 (RIGGS and BOTSTEIN 1987) could not be rescued by any of the mapping strains that we used. This failure was likely due to the presence of a second *amber* mutation elsewhere on the P22 genome in these strains. We believe the collection of mutants reported in Table 3 represents a *complete* set of the currently available, *well-behaved*, conditional

TABLE 2

Locations of endpoints in previously existing deletion mapping strains

Strain	Endpoint location
RIGGS and BOTSTEIN (1987) strain	
f223	Lies within interval BB or CC
f224	Defines interval T-U boundary
f226	Lies within interval Q or R
f227	Lies within interval P or Q
f229	Lies within interval Y or Z
f230	Lies within interval O or P
f236	Lies within interval R or S
f234, f235	Lies within interval QQ or RR
f228	Defines interval QQ-RR boundary
CHAN and BOTSTEIN (1972) strain	
DB123	Lies within interval R, S, T or U
DB5010	Lies within interval J
DB5059	Lies within interval II, JJ or KK
DB5060	Lies within interval HH or II
DB5061	Lies wtihin interval W or X
DB5062	Lies within interval P, Q or R

TABLE 3

P22 mutation locations from deletion mapping

Deletion interval[a]	Gene	Mutant allele[b]
B	*3*	*am*H1163[cd], *am*H1270[cd]
C	*3*	*am*H24, *am*H307, *am*H314, *am*H315, *am*H317[e], *am*H1038, *am*H1064, *am*H1080, *am*H1090, *am*H1113, *am*H1128, *am*H1129, *am*H1227, *am*H1282, *am*H1294, *am*H1301, *am*H1316, *am*H1349, *am*H1355, *am*H1358, *am*H1364, L *am*73, *ug*H7
D	*3*	*am*N6[e], *am*H14, *am*H30, *am*H309, *am*H322, *am*H329, *am*H1022, *am*H1056, *am*H1103, *am*H1251, *am*H1265, *am*H1277, *am*H1286, *am*H1288, *am*H1339, *am*H1340, *am*H1347, *am*H1415, *am*H1416, *am*H1431, *am*H1436, *am*H1438, *am*H1439, *am*H1441, *am*H1443, *am*H1449, *am*H1453, *am*H1456, *am*H1458, *am*H1461, *am*H1464, *am*H1467, *am*H1471, *ug*H6, *ug*H13, *ts*3.1
F	*3*	*cs*H135
H	*2*	*am*H1045[cf], *am*H1046[cf], *am*H1378[cf], *am*H1379[cf], *am*H1433[cf], L *am*19[f], *ts*4210
I	*2*	*cs*H22, *cs*H59, *cs*H92, *cs*H99, *cs*H144, *cs*H149, *cs*H157
J	*2*	*am*N16[ef], *am*H1307[f], *am*H1401[f], *am*H1425[f], *am*H1462[f], *cs*H82, *cs*H88, *cs*H89, *cs*H105, *cs*H118, *cs*H134, *cs*H162, *cs*H169, *cs*H170, *cs*H173
K	*2*	*am*H34, *am*H200, *am*H207, *am*H1024, *am*H1191, *am*H1222, *am*H1298, *am*H1359, *am*H1463[cf], *am*H1465, *am*H1466, *ts*N118, *cs*H104
L	*2*	*am*U210[f] *am*H303[f], *am*H320[f], *am*H1086[f], *am*H1094[f], *am*H1135[f], *am*H1154[f], *am*H1262[f], *am*H1275[e], *am*H1310[f], *am*H1312[f], *am*H1367[f], *am*H1377[f], *am*H1446[(d)f], *am*H1448[f]
M	*2*	*ts*2.1
O	*1*	*ts*1-3, *ts*14.1, *ts*N106, *cs*H129[g], *cs*H168[g]
P	*1*	*am*H201[e], *am*H313, *am*H1023, *am*H1107
Q	*1*	*am*N4[(f)], *am*H1210, *am*H1221, *am*H1297[cf], *am*H1352[cf], *ts*1.1, *ts*1-1, *ts*17.1, *ts*N101, *ts* su(*am*UT34)5, *ts* su(*am*Y232)11
R	*1*	*am*N32, *am*H44, *am*N101, *am*N124, *am*U207, *am*U212, *am*H1230, *am*H1285, *am*H1303
S	*1*	*am*H1160, *am*H1252[e], *am*H1309
T	*1*	*cs*H139
U	*1*	*am*H1445[f]
W	*1*	*am*N10, *am*N18, *am*N23, *am*N112, *am*U202, *am*H203, *am*U203, *am*U204[(d)h], *am*H205, *am*U205, *am*U206, *am*H1081, *am*H1097, *am*H1155, *am*H1178, *am*H1276, *am*H1278, *am*H1279, *am*H1314, *am*H1331

lethal mutations in the P22 region under study.

Most of the unpublished mutations had previously been tested for complementation against a standard set of *amber* mutations, and in nearly all cases those complementation results agreed with our deletion mapping results (BOTSTEIN, CHAN and WADDELL 1972; JARVIK 1975; J. KING, D. BOTSTEIN, M. SUSSKIND, H. PRELL, personal communications). We performed complementation tests with those few that disagreed with previous results and have in each case found those reports to be in apparent error (data not shown; see also footnote *g* in Table 3). We also performed complementation tests with all the previously untested mutants which fell into deletion mapping intervals that span gene boundaries. Mutations were thus found in 34 of the 45 intervals defined in Figure 1. The map interval locations for all of the mutations are given in Table 3. In all cases the physical location of the intervals containing mutations in the various complementation groups align perfectly with the open reading frames found in the nucleotide sequence of the region (Figure 1 and EPPLER *et al.* 1991). This set of mutants now provides a very accurate and substantial physical/genetic map of the region. In general our results agree with the previous ordering of a few of these alleles by GOUGH and LEVINE (1968), CHAN and BOTSTEIN (1972), BOTSTEIN, CHAN and WADDELL (1972), CHISHOLM *et al.* (1980), RUTILA and JACKSON (1981), WYCKOFF and CASJENS (1985) and RIGGS and BOTSTEIN (1987). We did, however, find several alleles, *1*$^{-}$ *am*N18, *1*$^{-}$ *am*N32, *1*$^{-}$ *am*H1034, *1*$^{-}$ *am*H1221, *1*$^{-}$ *am*H1230, *10*$^{-}$ *am*N107, *8*$^{-}$ *am*H1060, and *5*$^{-}$ *am*H1203, that in our map occupy clearly different positions than those previously reported. We have no explanation for these differences, but believe that our positions are accurate because of the redundancy and precisely known structure of our mapping strains.

The accuracy of the map generated above is good, in that we have determined the sequence alteration in

Deletion interval[a]	Gene	Mutant allele[b]
X	*1*	*am*H21$^{d(h)}$, *am*H58^{dh}, *am*H1034$^{(f)}$, *am*H1142$^{f(dh)}$, *am*H1211^{f}, *cs*rH21B, *cs*rH21D, *cs*rrrH21D3A
Z	*1*	*cs*H137
AA	*8*	*am*N123^{r}, *am*U237, *am*U239, *am*H1225
BB	*8*	*am*N26^{dh}, *am*H49, *am*N125$^{(h)}$, *am*H202^{r}, *am*H208^{h}, *am*U241, *am*U243, *am*H304$^{(h)}$, *am*H1060$^{(h)}$, *am*H1115^{h}, *am*H1136^{h}, *am*H1177, *am*H1234$^{(h)}$, *am*H1281^{h}, *am*H1284^{h}, *am*H1348^{r}
CC	*8*	*am*U238^{rdh}, *am*U240^{rdh}, *am*H1172$^{f(dh)}$, L *am*74, *ts*N102, *ts* su(*am*UT71)1, *ts*U172
DD	*8*	*cs*H167^{g}
EE	*5*	*am*N8^{h}, *am*N30, *am*U217, *am*U221^{h}, *am*U222$^{d(h)}$, *am*U223, *am*U224$^{(h)}$, *am*U225, *am*U230, *am*U235, *am*H312^{f}, *am*H1037^{h}, *am*H1055, *am*H1075, *am*H1151, *am*H1292^{h}, *am*H1318, L *am*78, *cs*H126^{g}
FF	*5*	*am*H1203^{fh}
GG	*5*	*ug*4, *ts*7
HH	*5*	*am*N114, *am*N122, amU213^{h}, *am*U214^{h}, *am*U216$^{(h)}$, *am*U218$^{d(h)}$, *am*U219, *am*U228, *am*U229, *am*U232, *am*U233, *am*U234, *am*H1169, *ts*3, *ts*8, *ts*N13, *ts*13.1, *ts*15.1, *ts*22, tsN105, *ts*N107, *ts*rH58E
II	*5*	*amN3*, *am*Y17, *am*H69, *am*N103, *am*N113^{h}, *am*U215$^{(h)}$, *am*U220$^{(h)}$, *am*U226, *am*U227^{h}, *am*H327, *am*H333, *am*H1353, *ts*5.1, *ts*6, *ts*10, *ts*N26, *ts*26.1, *ts*N53, *ts*rH137B, *ts*rH137C, *ts*rH137D
JJ	*5*	*ts*rH58G
KK	*5*	*am*N13^{h}, *ts*11, *ts*34, *ts*rH58H, *cs*rrH58G1
PP	*4*	*am*H1334, *am*H1368
QQ	*10*	*ts*11.1^{g}
RR	*10*	*am*N33^{rf}
SS	*10*	*am*H70^{f}, *am*N107, *ts*24.1

[a] Mapping intervals shown graphically in Figure 1. Those intervals which are not listed in this table contain no mutations.

[b] Mutations from the closely related phage L are so indicated.

[r] Serine inserting *amber* suppressor (*sup*D) is nonpermissive at 37°. Here and with other *amber* suppressor data, parentheses indicate variable poor suppression results–very tiny or no plaques depending upon precise plating conditions.

[d] Glutamine inserting *amber* suppressor (*sup*E) is nonpermissive at 37°.

[r] Sequence alteration known (EPPLER *et al.* 1991).

[f] Tyrosine inserting *amber* suppressor (*sup*F) is nonpermissive at 37°.

[g] Complementation tests, done both in liquid culture and on plates, with *ts* and *cs* mutations were not always unequivocal (JARVICK 1975; results not shown). It is thus possible that some missense mutations which fall into deletion intervals that span gene boundaries could have been assigned to the wrong gene. In each case we have listed these in the gene which the complementation test data most strongly indicated. Our tests with *ts*11.1 were particularly ambiguous, and it remains possible that it is a gene *4* mutation in interval PP, and csH129 and csH168 were not tested against the pSMV-1A plasmid.

[h] Leucine inserting *amber* suppressor (*sup*J) is nonpermissive at 37°.

nine *amber* mutants from this region (EPPLER *et al.* 1991), and all were found to lie within the interval into which they had been mapped. In addition, the sizes of amber fragments of gp*2* (H200, H1222), gp*1* (N10, H1081), gp*8* (N26, N125, H49, H202, H208, H304, H1281, H1284 and H1348) and gp*5* (N114) amber fragments, as measured in SDS-polyacrylamide electrophoresis gels, agree well with the map positions of these mutations (BOTSTEIN, WADDELL and KING 1973; KING, HALL and CASJENS 1978; YOUDERIAN and SUSSKIND 1980; our unpublished results). The map is fully internally consistent, and with very few exceptions the mapping of all mutations was unambiguous. However, the ability to form plaques for some *ts* mutations was very sensitive to the precise plating conditions and to the presence of pBR322 based plasmids in the host cell. Thus, the map positions of a few *ts* mutations, in particular *5*$^{-}$ *ts*7, *ts*rH137B and *ts*rH137C should be considered somewhat tentative at this time.

The suppressibility of each of the *amber* alleles was tested by measuring the ability of phages carrying them to form plaques on L plates at 37° with hosts containing isogenic *amber* suppressors that insert Gln, Ser, Tyr or Leu. The results are also indicated in Table 3. This information helps to identify mutations that occupy different sites within the various deletion intervals. In addition, incorrect amino acid substitutions are identified which may be of future use in the creation and analysis of missense proteins with partial function in DNA packaging.

DISCUSSION

The physical map: The physical map we have generated places 285 phage P22 and 4 phage L conditional lethal mutations onto a map containing 44 small

(<400 bp) intervals in the 5 genes required for P22 DNA packaging and 2 of the genes required for stabilization of packaged DNA within the head. The set of deletion mapping strains allows quick and accurate localization of new conditional lethal mutations in these genes to small, easily sequencable intervals. The map will certainly be of use in understanding new details in the function of the proteins that act during P22 DNA packaging. The reliability of the map is good, in that positive marker rescue is an extremely strong indication that the mutation lies within the P22 DNA of the plasmid from which the rescue is occurring, and the redundancy of the mapping strain set assures that much of the mapping information is positive in this sense. On the other hand, failure to find rescue from a plasmid could mean that the mutation lies within, but extremely near an end of the cloned P22 DNA fragment. Such a mutation would be placed on the wrong side of an interval boundary. If errors in mapping were made, they are likely of the form that could be explained by this type of failure to rescue. However, one of the *amber* mutations whose sequence is known, *8*$^-$ *am*H202, lies just twelve nucleotides from the pUS88 right endpoint, yet it is rescued unambiguously from this plasmid (see results). Thus, such mapping errors are no doubt rare, since the mutation would have to be less than twelve nucleotides from the end of the cloned DNA fragment to fail to show rescue.

In a few cases it is likely that we can predict the precise nucleotide change in mutations whose sequence alteration is not yet known. Hydroxylamine induced gene *3* mutations *am*H1363 and *am*H1270 in interval B, and gene *5* mutation *am*H1203 in interval FF, lie in intervals in which only one possible, single transition can give rise to an *amber* codon. In addition, these gene *3* alleles have identical, atypical suppression patterns suggesting that they are likely to be identical (Table 3). We predict that if these mutations are indeed transitions, they will affect codons 29 of gene *3* and 88 of gene *5*, respectively. Analogous logic places *5*$^-$ *ug*H4 at codon 145 of gene *5*.

Map saturation: Essentially all of the mutations on the map are thought to have arisen independently (see references in MATERIALS AND METHODS). The types of the mutations and the suppression patterns for the *amber* mutations (disregarding plaque size differences and temperature growth profiles for *ts* mutations) show that they represent a minimum of seventy-two different sequence alterations. The actual number of mutant sites is no doubt substantially larger; for example, among the nine sequenced *amber* mutations in this region, two, *8*$^-$ *am*H202 and *8*$^-$ *am*H1348, occupy different sites but are not separated by the above criteria (EPPLER *et al.* 1991). Nonetheless, the following arguments suggest that the mutageneses were not fully random. Hydroxylamine is known to induce transition mutations (TESSMAN, ISHIWA and KUMAR 1967), and indeed all five of the sequenced, hydroxylamine generated *amber* mutations are due to transitions (EPPLER *et al.* 1991). Only CAG and TGG among the sense codons can be changed to a TAG (*amber*) by a single transition. There are 131 (93 CAG and 38 TGG) such codons in the genes within the sequenced region under study. A small number of these, for example the last six in gene *1* (C-terminal to the *dif*1 mutation–see EPPLER *et al.* 1991), may lie in nonessential C-terminal regions of genes, and so *amber* mutations in those positions would not have been found. The 151 *amber* mutations in this study that were induced with hydroxylamine occupy a minimum of 33 sites (ignoring suppression phenotypes such as plaque size and taking into account that H202 and H1348 are known to be at different sites in interval BB–EPPLER *et al.* 1991), but the distribution does not appear to be random. For example, map interval C has only two in-frame TGGs and no in-frame CAGs, but contains 22 of the hydroxylamine generated *amber* mutations, and interval D contains four CAG/TGGs but contains 34 such mutations. Map interval BB contains eight CAG/TGGs, but of the seven hydroxylamine generated mutations in this interval that we tested by measuring the size of the amber fragment produced, four (H304, H1281, H1284, H1348) produced a 9-kD fragment and three (H49, H202, H208) produced a 14 kD fragment (data not shown), suggesting that they may occupy only two sites. However, the suppression phenotypes suggest that the class may be more heterogeneous. Finally, map intervals Y and OO each have six in-frame CAG/TGGs that have not been altered to TAG in any of the hydroxylamine generated mutations. It is clear that the transition generated *amber* mutations are quite far from saturating the possible sites, but hydroxylamine mutational hot spots would probably limit the usefulness of this type of mutagenesis of phage particles in any further attempt to find new *amber* mutations in this region of the P22 chromosome. Since they are not suppressed by a Gln inserting suppressor (and so are likely not derived from CAG), and the interval to which they map has no TGG codons, two of the hydroxylamine generated mutations, *1*$^-$ *am*H21 and *1*$^-$ *am*H58 are probably not single transitions.

Ramifications for P22 virion assembly: Some deductions about the function of the various proteins in P22 assembly which can be made from the current map are as follows: (1) The map shows that the proteins do not have large C-terminal dispensable regions, since mutations are found near the C termini. A fortuitous mutation in gene *1* has shown that at least the 50 C-terminal amino acids of the gene *1* protein are nonessential (EPPLER *et al.* 1991), but the *cs*H137 mutation (interval Z) shows that some residue within the C-terminal 109 amino acids is important for gp*1*

function. (2) Among the mutations mapped were several that were originally isolated as second-site revertants of mutations in other genes (JARVIK and BOTSTEIN 1975). These are *5*⁻ *ts*rH137B, *ts*rH137C and *ts*rH137D which were isolated as suppressors of *1*⁻ *cs*H137, and *5*⁻ *ts*rH58E, *ts*rH58G and *ts*rH58H which were isolated as suppressors of incorrectly (Gln) suppressed *1*⁻ *am*H58. These second-site suppressor mutations *may* indicate gp*1*– gp*5* regions of interprotein contact during prohead assembly (the C-terminal regions of both proteins), although other explanations remain possible. (3) The physical map will also allow us to begin to assign specific partial functions to different portions of the proteins involved in P22 DNA packaging. For example, BAZINET and KING (1988) argued that *8*⁻ *ts*U172 defines a site in gp*8* which interacts with the portal protein (gp*1*). This mutation lies near the center in the C-terminal half of the *8* gene. JARVIK and BOTSTEIN (1973) argued that the gene *2* defects caused by *ts*2.1 and *cs*H59 at nonpermissive temperatures occur at separable points in the assembly pathway, with the *ts*2.1 defect occurring before the *cs*H59 defect. Our map shows *ts*2.1 near the C terminus of gene *2* and *cs*H59 in the N-terminal quarter of the gene. They similarly found the *1*⁻ *cs*H137 defect to precede the *1*⁻ *ts*1.1 defect. Our map shows *cs*H137 in the extreme C terminus of gene *1* and *ts*1.1 in the N-terminal quarter of the gene. The detailed assembly defects caused by these four missense mutations are unknown at present. (4) Changes in the N terminus of the gene *2* protein and C terminus of the coat protein (gp*5*) appear to be particularly susceptible to giving rise to *cold-sensitive* and *temperature-sensitive* proteins, respectively. This may indicate that these regions are particularly critical in the folding-assembly process for these proteins (see for example FANE and KING 1987). (5) *Amber* suppressors are far from fully efficient, so all permissive *amber* mutant phage infected cells contain a substantial amount of *amber* fragment compared to full length protein. The map presented here shows that *amber* mutations can be isolated within the C-terminal one-third of at least genes *2*, *1* and *5* (and perhaps *8* and *4*), and in the cases tested, we know that the fragments are stable *in vivo* (KING, LENK and BOTSTEIN 1973; KING, HALL and CASJENS 1978; YOUDERIAN and SUSSKIND 1980; our own unpublished results). It thus appears that these fragments do not form partially functional, "assembly poisonous" proteins, which might add to the assembling structure but in so doing block further assembly and proper completion or function of the structure. This might be considered somewhat surprising in view of numerous recent observations that isolated protein domains often have partial activities relative to the full length proteins. However, if *E. coli* β-galactosidase synthesis is a reflection of typical prokaryotic translation, premature termination of normal translation appears to occur with a 50% probability for each one thousand codons decoded (MANLEY 1978; TSUNG, INOUYE and INOUYE 1989). Thus, for example, about one-quarter of normal translation starts on the P22 coat protein gene, which is 430 codons long, might be expected to result in the formation of an N-terminal fragment of coat protein. Thus, even in a wild-type infection a significant fraction of amino acids polymerized from the coat protein mRNA may be present as N-terminal fragments. It seems likely that many phage assembly proteins have avoided assembly poisonous function in these N-terminal peptides by being unable to fold or assemble without their C termini. It would be particularly advantageous for virus structural proteins to have evolved in this fashion because, due to the highly polymerized nature of the structures being built, a single poisonous fragment could in theory render a large number of normal protein molecules useless. It is interesting in this regard that the second-site revertants implicate the C termini of the coat and portal proteins in the assembly process (above), and that C-terminal gene *2* and *1* mutations appear to have earlier effects than N-terminal mutations in those genes (above). We have little direct knowledge about this aspect of phage assembly, but the T4 gene *11* protein C-terminal amino acid residues are known to be required for its assembly into baseplates (PLISHKER and BERGET 1984; BARRETT and BERGET 1989), and the C termini of the T4 gp*37* tail fiber polypeptides and possibly the P22 tail spike proteins are thought to initiate their multimerization (reviewed by CASJENS and HENDRIX 1988; FANE and KING 1987; SCHWARZ and BERGET 1989). Furthermore, there are very few documented cases where an *amber* fragment has been shown to be incorporated into an assembling bacteriophage precursor, although a great many analyses have been performed on structures made by *amber* mutants in phage structural protein genes. The only case we are aware of in which an *amber* fragment is incorporated into a structure is the T4 gene *48* encoded *amber* N022X fragment which assembles into phage baseplates (KIKUCHI and KING 1975). The gene *48* protein may be in the phage in an extended state, and so may not need to fold to bind to base plates (DUDA, GINGERY and EISERLING 1986). The map we have constructed and presented here will no doubt continue to be useful in future studies of P22 DNA packaging and prohead assembly.

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