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Molecular Biology of Bacteriophage Mu

Genetic and biochemical analysis reveals many unusual characteristics of this novel bacteriophage.

M. M. Howe and E. G. Bade

When sensitive strains of *Escherichia coli* K12 are infected with the temperate bacteriophage Mu, approximately 2 percent of the resulting lysogens are found to have acquired a new nutritional requirement (*1*). The unusual mutagenic capability of this bacteriophage stimulated much interest and prompted the name Mu (for *mu*tator). Since then, the original hypothesis that the mutagenesis occurs by insertion of the Mu prophage into the inactivated gene (*1*) has been confirmed by both genetic and physical means (*2–7*).

Since most temperate bacteriophages integrate into one or a small number of specific sites in the host chromosome, Mu is unusual in its ability to integrate in many sites. Much of the current work on Mu is directed toward understanding the mechanism by which this integration occurs.

Recent analysis has revealed that Mu is unusual in other ways as well. For example, Mu DNA contains host DNA sequences (*8–10*) and is found associated with host DNA during lytic development (*11–13*). It is interesting that in these aspects Mu seems to be more similar to the oncogenic animal viruses SV40 (simian virus 40) and polyoma (*14*) than to other bacteriophages.

This article includes a discussion of Mu integration, the properties of Mu-induced mutations, the genetics and physiology of Mu, and the structure and replication of the phage DNA.

General Properties of Mu

Mature Mu virions are similar in appearance to those of bacteriophage P2 (*15, 16*). The Mu virion is composed of a head 540 Å in diameter, a contractile tail sheath 1000 Å long and 180 Å wide, a base plate and tail spikes (*16*). The density of the mature particles was found to be 1.454 g/ml (*17*) and 1.468 g/ml (*16*) in independent measurements. As yet, nothing is known about the proteins of the virion. Information about the size and structure of the double-stranded DNA is presented below.

The host range of Mu is rather limited. It grows on *E. coli* K12 (*1*), on *Citrobacter freundii* (*18*), and on some strains of *Shigella dysenteriae* (*1*), but not on *Salmonella typhimurium* or on *E. coli* C, B, S, or W (*1*). Host range mutants of Mu able to grow on *E. coli* C and on *Shigella* have been isolated (*19*). Within *E. coli* K12 strains, some mutants resistant to Mu are also resistant to bacteriophages P1 and P2, and many show increased sensitivity to phages T3, T7, and C21 (*20*). The rate at which Mu adsorbs to sensitive cells varies depending on the conditions. In medium containing $5 \times 10^{-3}M$ to $1 \times 10^{-2}M$ Ca^{2+} and Mg^{2+} adsorption is 80 to 95 percent complete within 15 minutes at 37°C (*9, 19*).

The titers of Mu lysates often decrease with time. Part of this decrease is due to phage adsorption to cell debris remaining after brief centrifugation and can be prevented by more extensive centrifugation (*9*).

Upon infecting a sensitive bacterium, Mu may develop lytically to produce more phage or it may form a lysogen. In the lysogenic state the phage DNA is integrated into the host chromosome, and most functions of the prophage or of a superinfecting Mu are not expressed (*21, 22*). The formation of stable lysogens of Mu is not a very efficient process. In a single cycle of infection the majority of the cells are killed, and only 5 to 10 percent of the survivors are lysogens (*20*). The remainder, which are all sensitive to subsequent infection by the phage (*20*), may have been abortively lysogenized or simply not infected. The proportion of lysogens in a culture infected with Mu can be increased to 100 percent by prolonged incubation (for example, overnight) of the phage-cell mix-

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ture. In the latter case multiple cycles of phage infection result in the selective survival of the lysogens that are immune to superinfection.

Most Mu lysogens contain only a single Mu prophage; however, polylysogenic strains containing two or more prophages at different sites in the host chromosome also occur. The frequency of polylysogens is often high in a lysogenic population resulting from infection at high multiplicity (*20*).

Lytic development of Mu can be initiated by infection of sensitive strains or by heat induction of lysogens carrying specific mutant prophages (*11, 20, 23*) and proceeds with a similar time course in both cases (*23*). Such lytic growth results in the production of 50 to 100 PFU (*24*) per cell (*11, 20*), and in the production of a small number of generalized transducing particles (*25, 26*). The ratio of plaque-forming particles to total particles has not been determined.

During growth at 42°C the first intracellular phage particles can be detected at approximately 25 minutes after infection, with cell lysis occurring at 55 to 60 minutes, whereas at 32°C phage particles appear at 40 minutes with lysis at 70 to 75 minutes (*23*). The time course of Mu development is quite sensitive to growth conditions, in particular, to cell concentration; therefore, the times presented above may be increased by as much as 50 percent in different experiments (*9, 23*).

During Mu development host macromolecular synthesis is not appreciably shut off (*27*). Even late in the lytic cycle, synthesis of host-specific RNA (*21, 22, 28, 29*), DNA (*11, 12, 29*), and β-galactosidase (*9, 23*) continues.

During lytic development there is mutual exclusion between Mu and the lambdoid phages λ, 434, 21, and φ80 (*30*). A cell mixedly infected with Mu and one of these phages produces progeny of only one phage type (*30*). The mechanism of the exclusion is not known (*30*). In the lysogenic state Mu does not prevent the growth of any phages except superinfecting Mu (*20, 23*).

Mutations Induced by Mu

Mutagenesis by Mu is an efficient process. When sensitive bacteria are infected with Mu, 1 to 3 percent of the lysogenic survivors exhibit recognizable mutations (*1*). Within the surviving lysogenic population the mutation frequency in a single gene is 50 to 100 times higher than the spontaneous mutation frequency occurring without Mu infection (*1, 5, 20, 31*). This frequency of Mu-induced mutagenesis may be decreased somewhat if the gene is being transcribed at a high rate during infection. Mu-induced *lacZ* mutations [*lacZ*::Mu (*32*)] occur one-fifth as frequently when the lac operon is induced as when it is repressed (*10, 33, 34*). Perhaps the transcription apparatus covers the DNA and interferes with Mu integration.

Bacterial mating experiments (*1*) and P1 transduction (*2*) have shown that the mutation induced by Mu and the Mu prophage are genetically inseparable. It was, therefore, proposed that Mu-induced mutations are caused by linear insertion of the phage DNA into a gene, resulting in its inactivation (*1*). The prediction of this hypothesis, that the genetic linkage between two markers should decrease when a Mu-induced mutation occurs between them, has been confirmed in experiments involving conjugation (*3*), phage marker rescue (*4*), and the isolation of λ*gal* transducing phages (*5*). Analysis of DNA's containing Mu-induced mutations (as discussed later) also supports the hypothesis.

Although most Mu-induced mutations seem to be caused simply by the integration of Mu with no observable loss of host DNA (*33, 35*), Mu also causes deletion mutations (*23, 36*). An analysis of *lacZ* mutations isolated after Mu infection revealed that 10 percent were total deletions of *lacZ* and that none of the few deletion mutations tested were linked to a Mu prophage (*33*). A more recent examination of a large number of additional Mu-induced *lacZ* mutations showed that roughly 15 percent are deletions and that all are linked to a Mu prophage (*23, 36*). The contradictory prophage linkage data has not been completely explained but may be due to differences in host strain (*23*). Deletion mutations have also been found in the *strA* region of an episome after Mu infection (*37*). It is not yet known whether Mu-induced deletions occur in all regions of the chromosome and whether their formation is caused by the integration of Mu DNA or by some other process occurring in Mu-infected cells.

Much of the early work on Mu was concerned with studying the mutations it induced. In his first report (*1*) Taylor showed that Mu can cause various nutritional requirements reflecting the integration of the prophage into many different genes. Subsequent mapping of the Mu prophage in a large number of randomly isolated lysogens demonstrated that there were no preferred sites for integration and that the prophage could be located in many different regions of the chromosome (*23, 38*). Analysis of a large number of Mu-induced mutations in *lacZ* has shown that they map at many different sites even within this single cistron (*33, 35*). This evidence suggests but does not prove that Mu integrates without regard to host DNA sequence; however, it does require that any postulated specific host sequence be very short.

Mu-induced mutations are strongly polar (*4, 33, 35, 39*), probably because of a lack of production of messenger RNA distal to the Mu prophage (*40*). The mutations induced by Mu are extremely stable. In the few cases in which the reversion frequency was measured it was found to be less than 10^{-9} to 10^{-10} per colony-forming unit (CFU) (*1, 39, 41*). Although in some cases the absence of revertants may be due to an irreversible alteration of the mutated gene such as a deletion (see above), in most cases it simply reflects a lack of curing of the Mu prophage (*20*). The low frequency of curing has not been explained but might be due to lack of excision of the prophage, lack of rejoining of the chromosome ends after prophage excision, or death of the cell due to lytic development of the excised prophage.

A technique for isolating revertants of Mu-induced mutations at higher frequencies was recently found (*42*). This technique involves initial selection for heat-resistant survivors from strains carrying a heat inducible Mu prophage. Among the survivors of this selection are strains that carry defective Mu prophages, Mu *X*, and that exhibit frequencies of reversion of the Mu-induced mutation of up to 10^{-6} per CFU (*42*). The revertants appear to have lost the defective prophage (*42*). The relation between the process of reversion and normal prophage excision is not yet clear.

Mutants of Mu

The Mu prophage is not inducible by ultraviolet light or other agents known to induce a λ prophage (*3*). Since prophage induction is very useful in the study of temperate phages, one of the initial types of mutants isolated were those inducible by heat, namely the *cts* mutants (*11, 20*). These mutants, which produce turbid plaques at low temperature and clear plaques at high temperature, can lysogenize at low but not at high temperature. When such lysogens are shifted to high temperature, phage development is induced and results in the production of about 100 PFU per cell (*11, 20*). These mutants define a gene necessary for the maintenance of the prophage state and for the immunity of a lysogen to superinfection (*23*). Whether this gene encodes a repressor analogous to those of the lambdoid phages is not known.

Mutants that produce clear plaques and do not lysogenize at any temperature (*c*

mutants) have also been isolated. Complementation tests measuring the production of lysogens after pairwise mixed infections with the Mu *c* and Mu *cts* mutants have given contradictory results (*20, 23, 31*); therefore, the number of genes involved is not known. Deletion mapping (*43*) and three factor crosses (*44*) show that the *c* and *cts* mutations are located at one end of the Mu genetic map, termed the immunity end, close to gene *A* (Fig. 1).

Several hundred nonsense and temperature-sensitive mutants identifying 20 complementation groups, *A* to *S* and *lys* (Fig. 1), have been isolated (*43–49*). Phages defective in cistron *A* or *B* show little host killing or lysogenization (*43, 48*) and are deficient in DNA and late RNA synthesis (*21, 50*); thus cistrons *A* and *B* probably encode early functions involved in Mu regulation. Phages defective in cistron *C* synthesize phage-specific DNA but do not synthesize late Mu RNA (*51*). Mutants defective in *lys* produce a normal number of phage particles inside the cell, but show reduced cell lysis (*48*). Mutants in all other cistrons synthesize a normal amount of Mu-specific DNA (*50*) and may be involved in the morphogenesis of the phage particle (*51*).

Virulent mutants of Mu, Mu *vir*, which are able to grow in all Mu lysogens upon infection in liquid culture, have been isolated (*52*). Superinfection of a lysogen with Mu *vir* induces the development of the resident prophage and results in the production of a normal sized burst containing both prophage and superinfecting *vir* particles. The ratio of prophage to superinfecting phage types produced varies, depending on the lysogen infected: Single lysogens carrying the mutant prophage, Mu *gov* (growth of virulent), produce bursts with 50 to 70 percent Mu *vir* and 30 to 50 percent Mu *gov*, whereas multiple lysogens of Mu *gov* and single or multiple lysogens of Mu wild type yield 10 to 40 percent superinfecting *vir* and 60 to 90 percent prophage types. The reduced production of Mu *vir* from the latter group of lysogens may explain why Mu *vir* does not form visible plaques on lawns of those lysogens but does form plaques on lawns of single lysogens of Mu *gov* or of sensitive cells. The nature of the *gov* and *vir* mutations and their interaction is not yet known. The *gov* mutation seems to map to the right of gene *S*, and the *vir* mutations map near the immunity end (*52*).

If it is not properly modified, Mu DNA is restricted by the *E. coli* K12 and phage P1 restriction systems (*53*) [specific nuclease systems that degrade DNA recognized as foreign owing to its lack of specific nucleotide modification (*54*)]. Phages grown by lytic infection are restricted to an EOP (*24*) of 0.01 while phages grown by induction have an EOP of 0.5 (*53*). Mutants of Mu called *mom* (modification Mu) are more sensitive to restriction by these systems (EOP 10^{-4}) (*53*). These mutants transiently recover the EOP of *mom*$^+$ when grown in an induced Mu *mom*$^+$ lysogen (*53*). A likely explanation is that Mu *mom* mutants are defective in a DNA modification system that protects Mu DNA from restriction. The differences in plating efficiency of *mom*$^+$ phages grown by induction and infection might be due to differences in the synthesis or activity of this modification system under the two conditions. Deletion mapping and three-factor crosses place the *mom* mutation to the right of gene *S* (*53*).

Treatment of certain bacteriophages (for example, λ, T5, and T7) with chelating agents such as pyrophosphate inactivates the particles except for those having less DNA—that is, deletion mutants (*55*). Mutants of Mu resistant to treatment with pyrophosphate were originally considered to be deletion mutants (*56*), but subsequent work failed to demonstrate the presence of deletions in these phages (*9, 23, 57*). The lack of deletions in these mutants is understandable in the light of recent evidence which suggests that Mu packages a constant length or "headful" of DNA (*58*). By such a packaging mechanism any deletion would be compensated by an addition, and the resulting particles would have the same sensitivity to chelating agent as the wild type.

Genetic Map of Mu

Mapping of Mu amber mutants by two factor crosses during lytic growth resulted in a linear linkage map with genes *A* and *S* at opposite ends (*44, 49, 59*). In such vegetative crosses recombination within Mu is low, approximately 1 percent between *A* and *S* and is completely dependent on the bacterial *rec* (recombination) function (*44, 49, 59*). Therefore, Mu apparently does not encode an enzyme system which can effectively catalyze this type of vegetative recombination (*44, 49, 59*). Since Mu integrates at normal frequency in a *recA* host (recombination deficient) (*5, 60*), the integration system must be expressed in such a host. This lack of vegetative recombination in the presence of integration implies that the integration system cannot catalyze vegetative recombination (*49*).

The genetic map obtained by deletion mapping of Mu in the prophage state is the same for all Mu prophages and is the same as the vegetative map obtained from lytic crosses (*43–49, 59*). The prophage can be integrated in both possible orientations in a given operon. This was shown by prophage deletion mapping (*43, 44, 47*) and by analysis of Mu markers carried on various λd*pgl*Mu (defective λ transducing phages carrying one end of a Mu prophage originally integrated in gene *chlD* in the host chromosome close to the λ prophage) (*46*). A technique for easily determining the orientation of any Mu prophage has been developed. In it the direction of chromosome mobilization occurring as a result of recombination between a Mu prophage on the chromosome and a prophage on an F′ episome is measured. If the orientation of either of these prophages is known, the orientation of the second can be deduced from the direction of chromosome mobilization (*61*).

Low Level Prophage Induction

Exponentially growing cultures of Mu lysogens contain 10^5 to 10^6 PFU of spontaneously produced phage per milliliter. In cultures of some lysogens containing Mu integrated in *lac, lac*: : Mu (*32*), this level of free phage is increased 5- to 40-fold by induction of the lac operon with IPTG (*34, 38*). The inducibility of the prophages by IPTG is correlated with their orientation: only prophages whose *c* gene end is proximal to the lac operator are inducible (*61*). Among the inducible prophages the level of induction varies; for example, in double lysogens in which only one prophage is IPTG inducible, the level of induction is reduced to less than fivefold (*34, 38*). A similar orientation-dependent induction of the Mu prophage is also observed if Mu is integrated in a λ prophage which is then induced by ultraviolet irradiation (*62*).

The synthesis of *lac*- (*28, 40*) and Mu-specific (*27, 28*) RNA has been measured after IPTG induction of these *lac*: : Mu lysogens. The amount of lac RNA synthesized is proportional to the distance from the lac promoter to the Mu prophage (*27, 28, 40*). The synthesis of Mu-specific RNA is dependent on the orientation of the prophage (*27, 28*): with prophages which are not inducible by IPTG, there is no detectable Mu RNA synthesis; with prophages inducible by IPTG, the Mu RNA synthesized hybridizes to the heavy phage DNA strand, the same strand that hybridizes RNA made during lytic development (*21, 22*). A possible explanation is that transcription initiated in *lac* simply continues into the immunity and early region of Mu or temporarily displaces the presumptive Mu repressor resulting in phage development in a small number of cells.

Transduction

Lysates of Mu are capable of generalized transduction, that is, the phage-mediated transfer of host DNA fragments from one strain to another. The frequency of transduction varies between 10^{-6} and 10^{-9} transductant per PFU (*25*), with the highest frequencies being obtained for markers in the 0- to 1-minute region (*24*) of the *E. coli* K12 map (*26*). The transduced DNA fragment is substituted for the analogous region of the recipient chromosome in a process dependent on the recombination functions of the recipient (*25*). The observed levels of cotransduction of close markers suggests that the transducing particles contain primarily, if not completely, host DNA approximately one Mu DNA in length (*25*), but conclusive physical evidence has not yet been obtained. As will be discussed below, the mature Mu DNA molecule has at one end a segment of *E. coli* DNA (*8–10*). It is not yet clear whether this DNA can function as transducing DNA; however, it is not the only source of transducing DNA, since markers much too far apart to fit within this region can be cotransduced (*25*). All attempts to isolate specialized transducing phages of Mu have been unsuccessful (*25*).

Mu Promoted Integration of Non-Mu DNA

The λ*gal*$^+$ transducing phage λ*N*r14*gal*8 (*63*) is unable to replicate or integrate in *recA* su$^-$ hosts (*60, 64*). Simultaneous infection with Mu and this λ*gal* results in *gal*$^+$ transduction by the integration of the λ*gal* into the host chromosome between two similarly oriented Mu prophages (*60, 64*). The sequence of markers in the integrated λ*gal* can be any circular permutation of the λ map, and the Mu-λ*gal*-Mu structure can be integrated anywhere in the host chromosome. The integration is dependent on the expression of some Mu functions since it does not normally occur in strains already lysogenic for Mu. A model has been proposed in which the integration occurs by initial formation of a dimer Mu circle containing two functional attachment sites, followed by integration at one site into the circular λ*gal* and at the other site into the bacterial chromosome (*64*). Such dimer-sized Mu ring structures can be found early after Mu infection (*13*).

Integration of λ*gal* can also occur in Mu lysogens if the Mu prophage is partially induced, for example by infection with Mu *vir* or by growth of a heat inducible lysogen at partially inducing temperatures (*65*). As above, the integrated λ*gal* is located between two similarly oriented Mu prophages, but in this case the triple prophage structure is always found at the site of the original Mu prophage. The mechanism of integration in these cases is not yet understood, but a model consistent with the available data and with the previous model has been proposed (*65*).

Mutants defective in cistron *A* are unable to lysogenize and are also unable to integrate the λ*gal* during infection or partial prophage induction (*64*). Mutants in cistron *B* similarly do not lysogenize or integrate λ*gal* upon infection (*64*); however, they do integrate λ*gal* upon partial prophage induction (*65*). Plaque-forming mutants of Mu, designated Mu *nil* (no integration of λ) cannot integrate λ*gal* and are unable to form stable lysogens (*64*); however, they appear to be defective in prophage repression rather than in integration (*19, 66*).

Both F$^+$ and F′ factors can also be integrated into the chromosome of *recA* hosts, resulting in the production of Hfr's (*67*). This has been demonstrated by analysis of Mu-induced Hfr strains and by stimulation of chromosome mobilization (the ability of the chromosome to be transferred into an F$^-$ cell) during Mu development. The integration of F factors and λ*gal* probably occurs by the same mechanism, but transduction by λ*gal* requires both integration and stable lysogenization, while chromosome mobilization requires only integration. Thus, chromosome mobilization can be used to distinguish between mutants defective in prophage repression and those defective in integration.

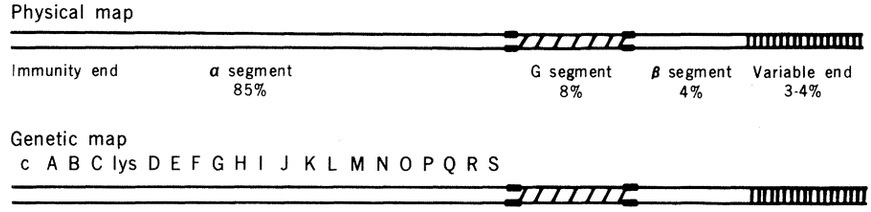

Fig. 1. Correlation of genetic and physical maps of Mu. This figure depicts the relationship of the genetic map of known essential genes to the physical map of the DNA which was derived by analysis of DNA homoduplexes. In the genetic map the position of the genes is intended to represent only relative gene order and not actual distances between genes.

The DNA of Mu

The DNA extracted from phage particles consists of double-stranded linear molecules (*6, 17*) for which no evidence of terminal redundancy has been reported. Measurements of the molecular size of this DNA by various techniques, for example, analytical centrifugation (*6*), viscosity (*17*), and electron microscopy (*6, 7, 10, 17, 68, 69*), have given values ranging from 22×10^6 daltons to 28×10^6 daltons. A value of 25×10^6 daltons is likely to be the most accurate since it was obtained in several laboratories from contour length measurements of the phage DNA made with the use of other phage DNA's of known molecular weight as internal standards (*7, 68, 69*).

Mu DNA is similar in composition to the DNA of its host, *E. coli* K12. By base composition analysis Mu DNA contains 48.1 percent G + C (*6, 24*). A value of 50 to 51 percent G + C has been calculated both from T_m determinations (*6*) and from the density in cesium chloride, 1.710 to 1.712 g/ml, as determined by equilibrium centrifugation (*6, 9*).

Modified bases have not yet been detected in Mu DNA (*6*). However, the existence of the Mu *mom* mutants which have increased sensitivity to restriction by P1 lysogens (*53*) and the variability in degree of cleavage of different Mu DNA preparations by purified restriction endonucleases (*70*) suggest that Mu DNA can indeed be modified. The degree of modification may depend on the method used to grow the lysate: Mu DNA prepared from phage grown by induction of a lysogen show fewer restriction sites than DNA preparations from phage grown by infection (*70*). The bases involved and the nature of the modification have not yet been determined.

The distribution of bases in the DNA of Mu is not uniform. After denaturation, the two strands can be separated from each other by the technique of poly(UG) binding and equilibrium centrifugation (*22*), originally used for phages lambda and T7 (*71*). The nonrandom distribution of bases also results in a characteristic pattern of strand separation loops, which arise within the double-stranded molecule when it is partially denatured (*72*). Electron microscope observation of denatured and reannealed populations of Mu DNA has revealed a characteristic heterogeneity, which has simplified the physical mapping of Mu genes (*56*) and allowed structural analysis of the integrated DNA (*7, 56, 73*). When Mu DNA is completely denatured and reannealed, the types of homoduplex

(*74*) molecules shown in Fig. 2 are produced (*7, 11, 22, 68, 73*). There are linear double-stranded molecules with nonrenaturing split ends at one end of the molecule, and linear double-stranded molecules with split ends and a nonhybridizing bubble, the G bubble (*75, 76*). We refer to the segments of the reannealed DNA as follows (see Fig. 2): the long double-stranded segment is called α, the short one β; the single-stranded segments between α and β constitute the G bubble, and the nonreannealing ends on the other side of β are called split ends. The region in native Mu DNA that, upon denaturation and reannealing, gives rise to the G bubble is called the G segment; the region forming the split ends is called the variable end and the opposite end is referred to as the immunity end.

The G bubble results from the reannealing of DNA strands whose G segments are in opposite orientations (that is, inverted) with respect to the rest of the DNA (*7, 70, 73*). Electron microscopic observation of denatured and partially reannealed preparations of Mu DNA reveals that there are homologous regions about 50 nucleotides long (*73*) at the junction of the G segment with both the α and the β segment (*10, 73, 77*). The inversion of the G segment probably occurs by recombination between these regions (*73*). Roughly 50 percent of the homoduplexes of DNA from phage grown by induction from a lysogen have the bubble structure (*28, 56, 68, 77*), whereas in DNA homoduplexes from phage grown by infection the frequency of G bubbles is lower and seems to vary with the multiplicity of infection (*56, 68*). This inversion also occurs in the prophage state (*7, 78*) and in recombination deficient *recA, B*, or *C* hosts (*7, 56*). Since inversion is independent of known host *rec* functions and is observed in defective Mu prophages that do not carry any of the known essential genes (*56, 70*), it is probably controlled either by phage functions encoded by DNA within or very close to the G segment or by an as yet unidentified host function. So far, aside from the possible location of functions *mom* and *gov* within or close to the G segment, no specific function has yet been attributed to it or to its intriguing behavior. The significance of the segments of internal redundancy within the G region (*73, 77*) which give rise to the hairpin structures is not clear.

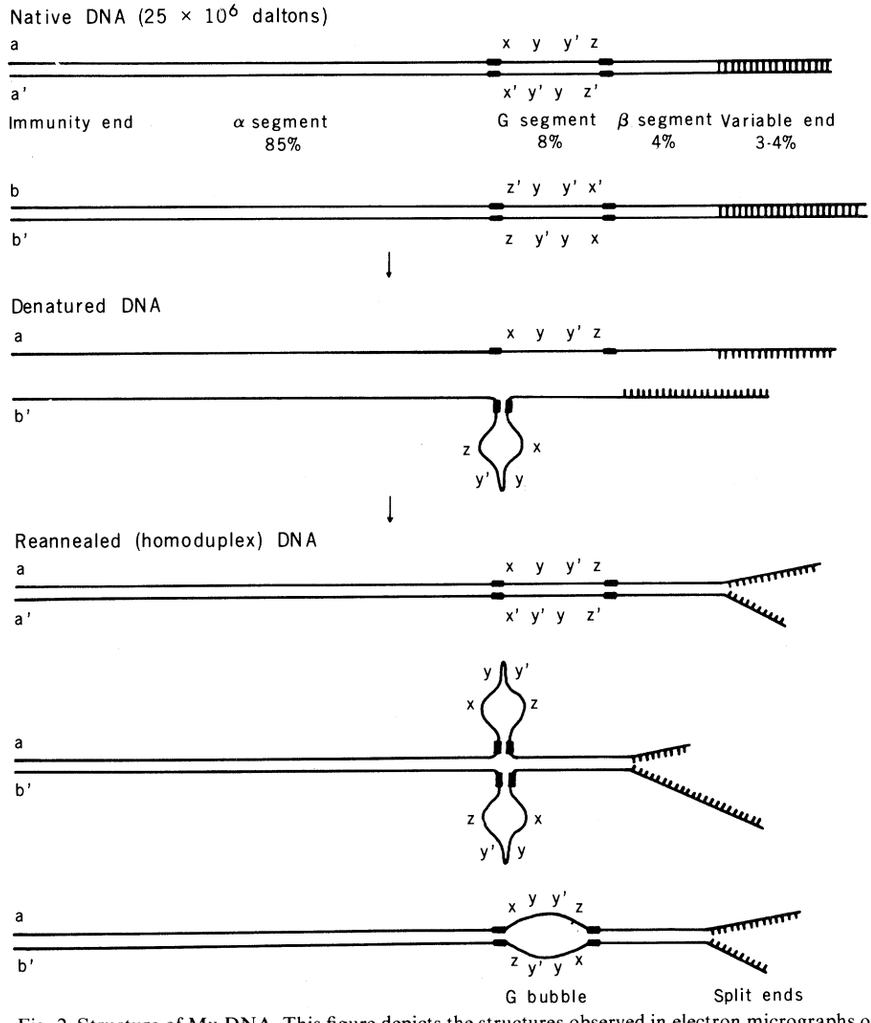

Fig. 2. Structure of Mu DNA. This figure depicts the structures observed in electron micrographs of denatured and reannealed Mu DNA. The *x y y' z* and complementary *x' y' y z'* lettering in the G segment does not represent specific genes but is intended to aid in the understanding of sequence relationships in the different forms.

The variable end of Mu DNA is present in all molecules, since all homoduplexes resulting from adequately denatured DNA contain split ends (*56, 68, 77*). This is true in all DNA populations, even those arising from single phage particles either by infection or by induction. These variable ends vary from 3 to 4 percent of the total length of Mu DNA (*68, 77*) and contain *E. coli* DNA (*8–10*). A mechanism for the generation of these variable end sequences has been proposed (*10, 12, 28, 58, 73*) and is discussed later.

The presence of host DNA sequences in the variable ends suggests that they could direct, by homology, the site of integration of Mu. This mechanism seems unlikely since phage grown on bacteria nonlysogenic for λ, or with a deletion of the *lac* region, or with a deletion at *tonB* are integrated with normal frequency into different genes of λ (*19*), of the *lac* operon (*23*), and of *tonB* (*57*), respectively.

Integrated DNA of Mu

It is well established now that most Mu-induced mutations are caused by linear insertion of Mu DNA into the gene inactivated. Genetic analysis has shown that there is a Mu prophage linked to almost every mutation induced by Mu. During conjugation the presence of a Mu-induced mutation in the Hfr delays the time of transfer of markers distal to the mutation by approximately 1 minute (*3*), the equivalent of about 25 × 10^6 daltons of DNA—a length roughly the size of a Mu prophage. Furthermore, isolation of a Mu-induced *lacI* mutation in an F′*lac* episome increases the contour length of that episome by approximately one Mu DNA length, slightly more than one Mu DNA length in one case (*6*) and slightly less in another (*7*). Since contour-length measurements of these large ring structures are relatively inaccurate, it is not advisable to use this approach to reach conclusions about additional effects of the insertion of Mu DNA, such as integration of the variable end, cointegration of other DNA, and the production of small deletions. Nevertheless, the facts that Mu can cointegrate other DNA molecules (*60, 64, 67*) and that the

prophage can be found substituted for large deletions of host DNA (*23, 36*) should always be kept in mind when analyzing DNA molecules into which Mu DNA has been inserted.

The analysis of triple DNA heteroduplexes of F′*lac*, F′*lacI*::Mu, and Mu also confirms that Mu is integrated linearly into F′*lac* and demonstrates that the prophage DNA is colinear (that is, not circularly permuted) with the DNA extracted from phage particles (*7*). This colinearity might prompt the hypothesis that both ends of the linear DNA extracted from phage particles correspond to the integration sequences of Mu DNA. This hypothesis is probably not correct since the variable ends of the integrating phage are not found in the prophage (*73*) and since, in the one case examined, a small piece of DNA found at the immunity end of the mature phage DNA is also missing from the prophage (*70*).

Correlation of the Genetic and Physical Maps of Mu

All the known essential genes of Mu map in the α segment of Mu DNA, with *S* being the closest to the G segment (Fig. 1). This was first demonstrated by analysis of DNA homoduplexes of a series of λd*pgl*Mu phages, defective λ transducing phages carrying the host gene *pgl* and one or the other end of a Mu prophage (*5, 46, 56*). These phages were isolated from lysates made by induction of several λ lysogens containing different Mu prophage insertions in the host gene *chlD* located next to *pgl*, which is very close to the λ integration site. The DNA homoduplexes of λd*pgl*Mu carrying Mu genes *c*, *A*, and *B* do not exhibit G bubbles, whereas those of λd*pgl*Mu carrying Mu genes, *P*, *Q*, *R*, and *S* do have G bubbles; therefore, the G segment is located near the *S* end of the Mu genetic map (*56*). Furthermore, the G segment can be located to the right of the known essential genes *A* to *S* because homoduplexes of a λd*pgl*Mu carrying the *S* end of Mu but none of the known essential genes still contain G bubbles (*56*).

The location of genes *A* to *S* in the α segment was confirmed in studies of bacterial strains containing only part of the Mu prophage (*66*). Strains containing structures $\alpha G\alpha$ or $\beta G\beta$ were obtained as segregation products resulting from recombination between G regions in two prophages which were integrated close to each other but with opposite orientations. The wild-type allele of amber mutations in genes *A* to *S* can be rescued from strains containing $\alpha G\alpha$ but not from those containing $\beta G\beta$. Both α and β are necessary for phage production; furthermore, they must be present in a contiguous $\alpha G\beta$ structure in order for phage production to occur. None of the strains containing one or both of the structures $\alpha G\alpha$ or $\beta G\beta$ are able to produce plaque-forming particles. As yet, no mutations have definitely been mapped within the G or β segments, although *mom* and *gov* may possibly be located within these regions (*52, 53*).

A further correlation of the physical and genetic maps is being accomplished by analysis of a series of λpMu transducing phages (also called λ-Mu hybrids), plaque-forming λ phages which contain a variable amount of Mu prophage DNA (*70, 79, 80*). They are isolated from lysates produced by induction of a λ*lac* prophage containing a Mu prophage integrated in the *lac* genes. The DNA fragments produced by cleavage of purified Mu and λpMu DNA with specific restriction endonucleases have been analyzed by gel electrophoresis. A comparison of the Mu DNA fragments present in different λpMu phages has allowed the derivation of a restriction fragment map of Mu (*70*). Correlation of the results of marker rescue experiments with the Mu DNA fragments present in the λpMu phages should allow the assignment of Mu genes to specific DNA fragments. Information locating genes *O* to *S* in specific fragments has already been obtained (*80*). It seems likely that the λpMu hybrid phages will be extremely useful, not only for this mapping but, more importantly, for studies of the integration and excision of Mu DNA and the function and regulation of various Mu genes.

RNA Synthesis

During lytic growth Mu is transcribed asymmetrically; more than 95 percent of the phage-specific RNA is complementary to the heavy DNA strand (*21, 22*). The direction of transcription is from the immunity end toward the variable end (*81*), but little is known about the size or possible polycistronic nature of the transcripts. The kinetics of RNA synthesis is bimodal (*21, 22, 29, 50, 81*). Within 2 to 5 minutes after infection, phage-specific RNA increases to 2 to 2.5 percent of the total pulse-labeled RNA. The amount of phage-specific transcripts then decreases at 5 to 10 minutes and gradually increases between 10 and 25 minutes (*21, 22, 29*). The fact that this decrease can be prevented by chloramphenicol suggests a negative control mechanism (*21, 81*). Massive phage RNA synthesis begins at 25 to 30 minutes and continues until lysis (*21, 22, 29*). Even at late times there is low level synthesis of early RNA (*9*). It is likely that the small amount of late RNA that hybridizes to the light strand is complementary to the invertible G segment (*21, 22, 81*). Since phage production is inhibited by the addition of rifampicin at any time during development in hosts sensitive to rifampicin and at no time in rifampicin-resistant hosts, it appears that Mu utilizes the host RNA polymerase throughout infection (*82*).

As will be described later, the time course of Mu DNA replication is similar to that of transcription (*13, 29*). It is not yet clear whether late RNA synthesis is dependent on replication, as is the case for T4 (*83*); an analysis of the effect of various amber mutations on these processes may help to answer this question. Mutants defective in genes *A* and *B* display a normal pattern of early transcription, but do not show the typical increase in RNA or DNA synthesis at late times (*21, 81*). Mutants of gene *C* are defective in late RNA but not DNA synthesis (*51*). Mutants defective in all the other known essential genes exhibit a wild-type pattern of DNA synthesis after heat induction of lysogens (*50*).

DNA Synthesis

Characterization of host and phage functions involved in the replication of Mu DNA has not yet been completed. Phage production does not require the host *dnaA* function but does require *dnaB*, *dnaC*, and probably *dnaE* functions (*84*).

The kinetics of DNA synthesis during lytic development has been determined by DNA-DNA hybridization (*29*) and by measuring the total incorporation of [^{3}H]thymine or thymidine into DNA in cells first treated with mitomycin C to suppress host DNA synthesis (*11, 13*). In cells treated with mitomycin C, DNA synthesis begins at approximately 25 to 30 minutes in a lytic cycle of 45 to 60 minutes (*11, 13*). When DNA synthesis occurring after heat induction of a lysogen is assayed by hybridization of pulse-labeled DNA to host and phage DNA, an increase in phage-specific DNA synthesis begins at 10 minutes and reaches a maximum at 25 to 30 minutes at which time a gradual decrease in host-specific synthesis begins (*29*).

The early events in Mu replication have been studied by analyzing the DNA extracted at various times after bacteria previously labeled with ^{3}H, ^{15}N, and ^{2}H were infected with ^{32}P-labeled phage of normal density (*13*). Within the first 10 minutes, part of the infecting phage DNA can be found in supercoils of two size classes: the majority are of Mu DNA size, while a

small proportion are roughly twice that size and are probably dimers. Slightly later (15 to 20 minutes), half the infecting phage DNA is found covalently linked to host DNA. Roughly one-third of this hybrid is in the form of supercoils, which vary in size over a wide range.

At later times during Mu development, when the rate of DNA synthesis is noticeably increased, the DNA forms present are linear molecules, relaxed rings, and supercoils (*12*). These supercoils vary continuously in size between the length of Mu DNA and up to five to six times that length, and do not occur solely in size classes corresponding to multiples of Mu DNA (*11, 12*). The population of supercoils contains both host (*12*) and phage DNA sequences (*11, 12*), with the relative proportion of phage sequences increasing later during the lytic cycle (*12, 29*).

Without the establishment of precursor-product relationships and a more complete analysis of the DNA forms present during Mu development, it is impossible to describe completely the process of Mu DNA replication; however, it may be worthwhile to discuss the features of the models proposed for this process (*11–13, 26*). It has been suggested that the linear infecting phage DNA forms ring structures which are integrated into host DNA (*13, 26*). Shortly after integration the phage DNA might excise along with pieces of host DNA to form larger rings containing both DNA's (*11, 13, 26*). These ring structures might then replicate, perhaps via a rolling circle type of replication intermediate, to produce additional copies of the phage and host DNA present in the ring (*12, 26*). During replication, the phage DNA might also excise from the rings (either completely or only partially) and reintegrate at random into other host DNA sequences (*65*). This process of continued excision and integration would result in the integration of the phage DNA into many different host sequences in a single cell. The resulting alternating phage-host DNA structure might then be a substrate for encapsulation by a mechanism in which the phage proteins recognize a site near the immunity end of Mu and package a "headful" of phage DNA including a small piece of host DNA at the immunity end and a large piece at the variable end (*12, 26, 58*). This "headful" packaging hypothesis is supported by recent evidence that shows that a small increase in the amount of DNA in the early region of Mu results in a compensating decrease in the length of the variable end (*58*). The generalized transducing particles might result from packaging of additional host DNA from the alternating DNA structures (*12, 26*). In order to explain the preferential transduction of markers in the 0- to 1-minute region of the *E. coli* map, it has been suggested that the phage DNA might associate somewhat preferentially with that region of the chromosome and that subsequent replication would result in an increase in the amount of that DNA available for packaging (*26*).

Excision

Processes involved in excision of Mu are poorly understood. In fact, it has not even been clearly demonstrated that excision, that is, the complete removal of Mu from the chromosome, actually occurs. The excision process has been studied by analyzing the effect of heat induction of a Mu *cts* prophage integrated in an F'*pro lac* episome. These studies suggest that induction results in cutting of the DNA near the immunity end of the prophage without subsequent resealing of the chromosome.

Heat induction of the episomal Mu *cts* prophage reduces episome transfer in mating approximately 100-fold (*85, 86*). Most of the episomes that are transferred still carry a Mu prophage and probably survive because of incomplete induction before mating (*85*). Induction results in concomitant loss of most of the supercoiled and relaxed circular forms of the episomal DNA molecules (*13*). The subsequent development of this DNA is obscured by the appearance of circular and superhelical molecules generated by Mu growth. The amount of host DNA removed with the Mu prophage and the fate of the remaining host DNA are not yet known.

Heat induction of a Mu *cts* prophage located on the chromosome causes very little decrease in episome transfer or in the amount of circular episomal DNA, unless there is also a prophage on the episome (*13, 85, 86*). The episomal DNA may be cut near the immunity end of the prophage since episomes deleted for the prophage *S* end are cut whereas those deleted for the immunity end are not cut after heat induction of the chromosomal prophage (*13*). This scission near the immunity end of the prophage may explain the absence of transduction of host markers near that end of the prophage in lysates made by induction (*23*).

Overview of the Integration Process

The mechanism by which Mu integrates is not yet understood; however, there are hypotheses that are consistent with the known features. The DNA of the infecting phage particle is linear and contains heterogeneous host DNA sequences at both ends (*8–10*). It has been postulated that this infecting DNA enters the cell and then circularizes (*13, 26*). Recent observation of the formation of Mu-sized ring structures from infecting DNA (*13*) lends support to this hypothesis. It is not known if these rings contain the host DNA segments; however, the heterogeneity of those sequences (*9, 56, 68, 77*) and their absence from the prophage (*73*) make it likely that they would be lost during ring formation.

Since the order of phage genes is the same in every Mu lysogen (*43–49, 59*), there probably is an attachment site on the phage chromosome where the integrative recombination event occurs. Furthermore, the evidence that the mature phage DNA and the prophage DNA are colinear (*7*) suggests that the attachment site is near the ends of the DNA or is generated by the circularization process (*65*).

Integration of the circularized phage DNA might then be accomplished by reciprocal recombination of the phage attachment site with the host chromosome. Since stably integrated Mu prophages are found randomly distributed over the host chromosome (*1, 23, 33, 35, 38*), it seems likely that this initial integration event would also occur at random. It is also likely that the integration normally occurs without loss of host DNA sequences because usually all host markers are present in lysogens (*35*) and because, under the appropriate conditions, most Mu-induced mutations can revert (*42*).

The involvement of host and phage functions in the integration process has not been studied extensively. Integration, as measured by the formation of Mu-induced mutations and stable lysogens, does occur in a variety of recombination and repair deficient hosts (*3*). As yet, there are no host functions known to be required for the integration process.

Phage-specific functions are needed for integration. Infecting phages defective in genes *A* or *B* do not form lysogens (*43, 48*), do not promote the integration of λ*gal* (*64*), and do not stimulate chromosome mobilization (*67*). However, the *A* and *B* mutations are highly pleiotropic in that they abolish late RNA and DNA synthesis as well as integration (*21, 50*). The effect of the mutations on the integration process alone, for example on the formation of ring structures, has not been examined. There is some evidence that *B* mutants still may express part, if not all, of a functional integration system; partial induction of a *B*-defective prophage can stimulate the integration of λ*gal* into the chromosome, whereas partial induction of an *A*-defective prophage does not (*65*).

As yet, there are no plaque-forming integration-deficient mutants of Mu. Perhaps the mutants are rare and simply have

not been found, or perhaps phages defective in integration are unable to grow. The latter explanation seems possible since replication and encapsulation appear to occur while Mu is in an integrated form (*11, 12, 58*).

Uses of Mu

The previous discussion has focused on the properties of Mu and its development. It is also worthwhile to consider how Mu can be employed as a tool.

1) As a mutagen Mu produces a defined type of insertion mutation which is not leaky and is very strongly polar. This property has been used to study colicin induction (*87*), to locate the late promoter of λ (*4*), and to determine the cistronic organization and direction of transcription of ribosomal protein genes (*37, 88, 89*), of flagellar genes (*90*), and genes involved in nucleoside and deoxynucleoside catabolism (*91*). In such studies it is essential to distinguish between mutations caused simply by Mu integration and those that involve deletions of host DNA as well (*37, 89*).

2) Under normal conditions Mu-induced mutations are nonreverting (*1*) and are, therefore, useful for the isolation of secondary site mutations that alter the original mutant phenotype (*2*).

3) Mu is ideal for use as a constant DNA sequence at different positions on the chromosome. The origin and direction of DNA replication of the *E. coli* chromosome have been analyzed by measuring the time of replication of Mu prophages located in different segments of the chromosome relative to a λ prophage integrated at its normal site (*92*). Fusion of *lac* genes to the *ara* promoter has been accomplished by integrating Mu into the operons to be fused, with the use of Mu-Mu homology for the recombination event that brings the two genes close together, and then selecting for deletions of the Mu *cts* prophage to accomplish the fusion event (*93*).

4) The polarity of Mu-induced mutations may facilitate the isolation of messenger RNA fragments of defined lengths extending from the promoter to the Mu prophage (*40*).

5) The analysis of DNA heteroduplexes of episomes containing a Mu prophage in known host genes allows the physical mapping of those genes (*7*).

6) Mu can be used as a generalized transducing phage (*25, 26*); however, the low efficiency of transduction and small size of transducing DNA make it less useful than bacteriophage P1.

7) Mu can be used to generate deletions. (i) About 15 percent of the known Mu-induced mutations in *lacZ* are deletions (*33, 36*). (ii) If Mu *cts* is integrated close to a gene of interest, deletions in that gene can be isolated by selection for simultaneous loss of the killing functions of the prophage at high temperature and the function of the nearby gene (*41, 42, 45, 46*). A procedure is possible whereby Mu *cts* is inserted directly into the gene in which the deletions are desired and then a single selection is made for heat resistance, but this method is more difficult because of the predominance of nondeletion types of mutation to heat resistance (*24*). (iii) Specific deletions of host DNA can be generated by insertion of Mu prophages on both sides of the region of the chromosome to be deleted and isolation of the deletion strain arising by recombination between the two prophages (*76*). (iv) Phages containing internal deletions have been isolated among the rare plaque-forming phages produced by induction of a λ prophage containing a Mu prophage integrated in it (*68, 77*).

8) The use of Mu to direct the integration of episomic elements in both a random and a specific way is almost unlimited. The random Mu-promoted integration of both λ*gal* (*57, 62*) and F factors (*65*) into the chromosome has been described. It appears that the only requirements for this type of integration are the expression of some functions of Mu and the circularity of the DNA to be integrated. No case of integration of linear DNA has yet been reported.

The integration of an episomic element at a specific site can be accomplished by inserting one Mu prophage at that site and one on the episome and then integrating the episome into the chromosome by recombination between the two Mu prophages (*58, 65, 94*).

These techniques have been used to generate Hfr's in *Citrobacter freundii* (*18*), to generate Hfr's with opposite orientations at specific sites in *E. coli* (*58, 65*), and to generate transpositions of host DNA and allow construction of new episomes (*95*).

Outlook

Since the discovery of Mu in 1963, much has been learned concerning the mechanism of mutagenesis by insertion of the Mu prophage into the inactivated gene. The continuing study of Mu should help to further elucidate this unusual recombination process and to allow the description of other characteristics of Mu development as well.

There are three other areas of interest which will certainly receive attention in the near future. The first is the relation of Mu to other insertion elements such as the *E. coli* I S sequences (*96*) and drug resistance determinants (*97*). The second is the possible use of Mu as a model system in the study of oncogenic viruses. This use is prompted by the observed similarities in DNA structure and replication of Mu, SV40, and polyoma virus (*8–14*). The third is the use of Mu for various types of genetic engineering in bacterial systems with potential for future use in eukaryotic systems as well.

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24. Abbreviations: PFU, plaque-forming unit; CFU, colony-forming unit; EOP, efficiency of plating; IPTG, isopropyl-β-D-thiogalactopyranoside; G + C, guanine plus cytosine; poly(UG), polyuridylate-guanylate; T_m, melting temperature; 0 to 1 minute means that region of the circular 90-minute linkage map of *E. coli* K12, as defined by A. L. Taylor and C. D. Trotter [*Bacteriol. Rev.* **36**, 504 (1972)]; *lac*, *ara*, and *gal*, genes for catabolism of lactose, arabinose, and galactose, respectively; λ*gal* and λ*lac*, lambda transducing phages carrying host *gal* or *lac* genes; *strA* ribosomal protein, mutants are resistant to streptomycin; *chlD*, gene for nitrate reductase—mutants are resistant to chlorate; *pgl*, gene for 6-phosphogluconolactonase; *pro*, gene for proline biosynthesis; *tonB*, receptor for φ80 and colicins B, I, V—mutants are resistant to φ80 and the colicins mentioned; *recA*, gene required for genetic recombination—mutants are recombination deficient; su⁻, lack of suppressor activity; *lys*, gene for Mu lysis function; F⁺, fertility factor resulting in the ability of strains to act as donors in conjugation; F′, fertility factor carrying specific host genes; Hfr, strain in which the fertility factor is integrated into the chromosome, leading to high frequency transfer of chromosomal genes during conjugation.
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32. The following notation system was designed and accepted by participants at the Mu Workshop held at Cold Spring Harbor Laboratory, New York, July 1972:
1) *Mu genotype.* Amber, Mu-1 *Aam*1093; clear, Mu-1 *cts*62, Mu-1 *c*20; temperature sensitive, Mu-1 *Ats*1720; absolute defective, Mu-1 *Adef*1721. In order to avoid possible assignment of the same allele number to different mutants in different laboratories, blocks of allele numbers were assigned to the following investigators: 1000 to 1999, M. Howe; 2000 to 2999, P. van de Putte; 3000 to 3999, A. Toussaint; 4000 to 4999, J. Abelson; 5000 to 5999, A. Bukhari; 6000 to 6999, A. Taylor. Exceptions to this numbering system are the already commonly used clear mutants *cts*4, *cts*61, and *cts*62.
2) *Lysogens.* Mu lysogen, K12 (Mu-1); lysogen containing known independent prophages, K12 (Mu-1) (Mu-1); lysogen with unknown number of prophages greater than one, K12 (Mu-1) (*n*); Mu integration in a gene, *proA*, proved by linkage of prophage to the mutation *proA*23 : : (Mu-1); Mu integration in gene presumed due to frequency of mutagenesis but not proved by linkage, *proA*23 : ? : (Mu-1); notation in text for Mu induced mutation in *proA*, *proA* Mu+.
3) *Orientation of the Mu prophage.* The notation given above is sufficient for cases in which the orientation of the Mu prophage is not known. If the orientation of the prophage is known, it should be indicated by a + sign or − sign before the Mu genotype—for example, *proA*23 : : (+ Mu-1). A + orientation means that if one moves around the *E. coli* map in a clockwise motion, the immunity end of the Mu prophage will be reached first and the *S* end last. The orientation of Mu on an episome is to be expressed as if the Mu were transposed onto the chromosome.
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75. The nomenclature used here is an attempt to unify the existing terms (*7*, *11*, *12*, *22*, *56*, *68*, *73*) with the nomenclature recommendations for DNA duplex structures of Davidson and Szybalski (*76*). This nomenclature should avoid some of the frequent confusions with respect to the fact that the split end and the bubble structures do not exist as such in native DNA but can be seen only after adequate denaturation and reannealing of Mu DNA preparations.
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