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Isolation and Mapping of Mu *nu* Mutants Which Grow in *him* Mutants of *E. coli*

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Mu *nuA* and Mu *nuB* mutants were selected by their ability to form plaques on lawns of *Escherichia coli himA* and *himB* mutants, respectively. Deletion mapping of the *nuA* and *nuB* mutations by marker rescue from λpMu transducing phages or from hosts containing deleted Mu prophages indicated that *nuA* mutations are located within or to the left of gene *A* and that *nuB* mutations are located within or between Mu genes *G* and *I*.

One approach to defining the interactions between a phage and its bacterial host is to isolate and characterize host mutants that inhibit some aspect of phage development and then phage mutants which overcome that inhibition. For temperate phage Mu, *Escherichia coli* K12 *dnaB*, *dnaC*, and possibly *dnaE* functions are needed for phage replication (*1*), and host RNA polymerase is required for phage transcription (*2*). Mu also requires host functions for protein synthesis and energy metabolism. Other host functions essential for Mu development have not yet been well characterized.

Mutants of *E. coli* (*him*) which prevent integration of phage lambda into the host chromosome were isolated by their ability to survive infection with a λ N^- *int*c phage which kills cells in which it integrates (*3*). Strains containing *himA* mutations, which are located at 38 min on the *E. coli* map (*4*), are defective in λ integration for two reasons. They produce reduced levels of λ integrase protein (*5*), and they are defective in one subunit of the integration host factor which participates directly in the λ integration reaction (*6*). The *himB* mutation was originally isolated as a *himB himC* double mutant and is located at 82 min in or near *gyrB*. It does not block normal λ integration or excision but is defective for λ excision from a secondary *att* site (*4*).

The *himA* and *himB* mutations also inhibit the growth of Mu (*4*). Mu development is severely inhibited in the *himA* host resulting in the production of very few phage and little host cell death (*7*, R. K. Yoshida and M. M. Howe, manuscript in preparation). In contrast, in the *himB* host Mu growth is only partially inhibited, resulting in normal cell death and the generation of pinpoint plaques arising by delayed production of the phage burst (R. K. Yoshida and M. M. Howe, manuscript in preparation). To help determine the mechanism of growth inhibition in these hosts we mutagenized lysates of the heat inducible phage Mu *cts*62 (*8*) by growth (*9*, slightly modified) in the *mutD* host KD1067 (*10*) and selected for Mu *cts*62 *nuA* mutants able to form plaques on lawns of a *himA*42 host (*4*) and Mu *cts*62 *nuB* mutants able to form large plaques on lawns of a *himB*114 host (*4*) on TCMG (*11*) plates at 37°. Both *nuA* and *nuB* mutant phages arose at frequencies of 10^{-6} to 10^{-9} per plaque-forming phage.

The *nu* mutations were located on the

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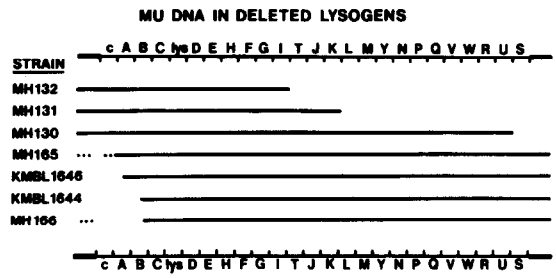
genetic map of Mu by deletion mapping by marker rescue from λpMu transducing phages (*12*) and from hosts with partially deleted Mu prophages (*8*). Since rescue of the nu^+ allele was not directly selectable, amber mutations were introduced into the Mu *nu* phages by recombination or *mutD* mutagenesis (*9*); then in the marker rescue assay am^+ recombinants were selected and scored for corescue of the nu^+ allele.

The results shown in Fig. 1 demonstrate that *nuA* mutations are located within or to the left of gene *A* at two distinguishable sites. The wild type alleles of the *nuA*1 and *nuA*3 mutations can be rescued from prophage deletion strain MH165 (*8*) but not from strain KMBL1646 (*13*) whereas the wild type alleles of *nuA*106 and *nuA*107 cannot be rescued from either strain. The absence of nu^+ rescue from strain KMBL 1646, which carries a deletion ending between *A*1504 and *A*1093 indicates that the *nuA* alleles are located to the left of *A*1093; however the absence of knowledge concerning the precise location of either endpoint of the deletion in strain MH165 makes it impossible to locate the mutations more definitively from this data.

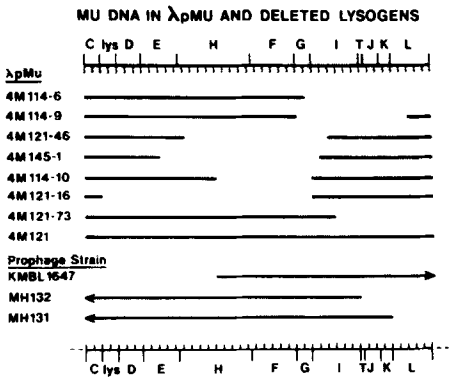
The results of deletion mapping of the *nuB* mutations by marker rescue from λpMu transducing phages (*12*) and prophage deletion strains (*8*, *12*) (Fig. 2) demonstrate that the *nuB* mutations are located in the late region of Mu within or between Mu genes *G* and *I*. The wild type



PERCENT CO-RESCUE OF nu^+ WITH am^+ ALLELE

STRAIN	MU nuA1 E1006	MU nuA3 F7605	MU nuA106 E7626	MU nuA107 D7631
MH132	100 (48)	90 (48)	73 (48)	57 (46)
MH131	---	96 (48)	87 (48)	52 (46)
MH130	---	96 (48)	100 (48)	91 (46)
MH165	11 (719)	19 (48)	< 0.2 (432)	< 0.2 (506)
KMBL1646	< 0.2 (432)	< 1.4 (70)	< 1.0 (96)	< 0.3 (322)
KMBL1644	< 2.1 (48)	< 2.4 (41)	---	---
MH166	< 2.1 (48)	< 3.6 (28)	---	< 0.7 (138)

FIG. 1. Deletion mapping of *nuA* mutations. The genetic map of the Mu prophage with genes *c* through *U* is given at the top left of the figure. The Mu DNA remaining in each prophage deletion strain is indicated by the horizontal line below the map. The dotted lines indicate that the exact endpoints of the deletions are unknown. The thick lines at the ends of the Mu DNA represent host DNA sequences immediately adjacent to the prophage. The reported locations of deletion endpoints in the MH (*8*) and KMBL (*13*) strains were based on marker rescue with only a few mutations in each gene. More detailed marker rescue analysis with amber mutants described by O'Day *et al.* (*12*) showed that the deletion endpoint in KMBL1646 was located between *A*1504 and *A*1093 and the endpoint in KMBL1644 was located between *B*5150 and the pair *B*5175 and *B*5176. The rightmost deletion endpoint in strain MH165 was located to the left of the leftmost *A*am mutations. (1791 and 7302); however, the locations of the leftmost deletion endpoints in strains MH165 and MH166 are unknown. They were originally selected as trimethoprim-resistant mutants to isolate deletions between *thyA* and a Mu inserted in *lys* (*8*); however, since they revert to thy^+ they do not contain such a deletion. Thus, their left deletion endpoints are unknown and could be within either Mu or host DNA. Two amber mutant derivatives of each of 4 Mu *cts*62 phages carrying independently isolated *nuA* mutations were used to locate the *nuA* mutations by selecting for rescue of the am^+ marker and scoring for corescue of the nu^+ allele. Spot marker rescue assays (modified from *14*) were performed, and the resulting areas of lysis were resuspended in SM diluting fluid (*8*) and plated for single plaques on lawns of the Su^- host on TCMG (*11*) plates at 37°. Individual am^+ plaques were resuspended in SM and spotted onto lawns of strains K37 (him^+) and K634 (*himA*42) (*4*) on TCMG plates which were scored for phage growth after overnight incubation at 37°. The values given in the right portion of the figure indicate the percentage of am^+ recombinants which also rescued the nu^+ allele for one representative amber mutant for each phage. Numbers in parentheses indicate the total number of am^+ recombinants tested. Dashes indicate that the particular phage/host combination was not tested.



PERCENT CO-RESCUE OF nu^+ WITH am^+ ALLELE

	Mu nuB1		Mu nuB2		Mu nuB103		Mu nuB105	
	F7609	L7613	F7620	J7621	F7625	I7624	lys7633	K7635
4M114-6	<0.8 (133)	---	<1.0 (96)	---	<1.0 (96)	---	<0.1 (799)	---
4M114-9	---	---	<2.1 (48)	---	---	---	0.2 (476)	---
4M121-46	---	<1.4 (72)	---	<2.1 (48)	---	---	<1.0 (96)	<0.6 (176)
4M145-1	---	<0.7 (143)	---	<1.1 (94)	---	<1.0 (96)	<0.1 (960)	<0.2 (479)
4M114-10	---	---	---	---	---	---	<0.1 (1051)	---
4M121-16	---	5.6 (72)	---	7.3 (96)	---	6.2 (96)	---	5.0 (240)
4M121-73	13 (16)	---	13 (24)	---	40 (96)	---	14 (96)	---
4M121	71 (48)	54 (48)	---	65 (48)	---	75 (48)	14 (96)	---
KMBL1647	94 (48)	38 (48)	73 (48)	21 (48)	94 (47)	67 (48)	---	31 (48)
MH132	60 (30)	---	71 (48)	---	77 (48)	---	37 (30)	---
MH131	---	---	---	48 (48)	---	---	---	60 (48)

FIG. 2. Deletion mapping of *nuB* mutations. The top left portion of the figure depicts the map of the central region of Mu (*12*) containing genes *C* through *L*; each small subdivision represents one deletion interval and the large subdivisions indicate the genes. The Mu DNA carried by each λpMu transducing phage (*12*) or prophage deletion strain (*8, 12*) is indicated by the horizontal lines. The arrows for the prophage deletion strains indicate that the strain contains additional Mu DNA in the direction to which the arrow points. Two amber mutant derivatives, one on each side of the *G–I* region, were isolated for each of four independently isolated Mu *cts62 nuB* mutant phages and were used as described in the legend to Fig. 1 for am^+ spot marker rescue analysis followed by scoring for corescue of the nu^+ allele by growth on strain K760 (Him$^+$ Su$^+$ *dctA ilv his pps gal strA*) but not on strain K761 (*himB*114 Su$^+$ *dctA his pps gal strA*). The method of presentation of results is described in the legend to Fig. 1.

alleles of all four *nuB* mutations were rescued from λpMu 4M121-16 but not from λpMu 4M145-1. λpMu 4M121-16 is deleted for wild type alleles of all known *G* amber mutations but allows rescue of all *I* amber mutations; while λpMu 4M145-1 is deleted for the DNA corresponding to all *G* amber mutations and the adjacent 6 *I* mutations (12).

Marker rescue of the *nuB*105 phage with λpMu 114-9 gave the unexpected result of 0.2% rescue (nu^+/am^+) despite the absence of the *G–I* region in the latter phage. This was based on finding 1 nu^+ plaque among 478 am^+ recombinants but was not confirmed in tests of 1143 additional am^+ recombinants which were all *nuB*. Examination of the results with other phages, particularly with λpMu 114-6 which carries even more Mu DNA than λpMu 114-9 but still does not allow rescue of nu^+, led us to disregard the single plaque observed with λpMu 114-9.

The location of *nu* mutations is consistent with the properties of Mu in the *him* hosts. Mu development in the *himA* host is blocked early before cell killing can occur (*7*) and the *nuA* mutations are located in the region of Mu which is transcribed early (*15*). Similarly, Mu growth in the *himB* host causes normal levels of cell death and a delayed phage production (R. K. Yoshida and M. M. Howe, manuscript in preparation), presumably by inhibiting a later step in phage development, and the *nuB* mutations are located in a region transcribed later. The precise nature of the block to Mu development in these hosts is not yet clear. Mu integration in the *himA* host is normal (*7*; R. K. Yoshida and M. M. Howe, manuscript in preparation) but DNA synthesis (B. Waggoner and M. Pato, personal communication) and late RNA synthesis (R. K. Yoshida and M. M. Howe, manuscript in preparation) are blocked. The *himA* gene product might participate directly in Mu DNA replication and only indirectly affect late transcription, or conversely, it might be necessary for transcription of genes and/or sites which are required for replication. Alternatively, transposition of Mu subsequent to the initial integration event might require *himA* gene product and thus result in the requirement for *himA* for DNA replication. If the *himB* mutations are altered in one of the subunits of DNA gyrase, as is suggested by their linkage to *gyrB* (*4*),

they might exert their effect on Mu via altered transcription, delayed replication, or delayed maturation of the phage particles. Further experiments to define the roles of *himA* and *himB* in Mu development and the alterations present in the *nuA* and *nuB* mutants are in progress.

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REFERENCES

1. TOUSSAINT, A., and FAELEN, M., *Molec. Gen. Genet.* **131,** 209-214 (1974).
2. TOUSSAINT, A., and LECOCQ, J., *Molec. Gen. Genet.* **129,** 185-188 (1974).
3. MILLER, H. I., and FRIEDMAN, D. I., *In* "DNA Insertion Elements, Plasmids and Episomes" (A. I. Bukhari, J. Shapiro, and S. Adhya, eds.), pp. 349-356. Cold Spring Harbor Laboratory, New York, 1978.
4. MILLER, H. I., KIKUCHI, A., NASH, H. A., WEISBERG, R. A., and FRIEDMAN, D. I., *Cold Spring Harbor Symp. Quant. Biol.* **43,** 1121-1126 (1979).
5. MILLER, H. I., *Cell* **25,** 269-276 (1981).
6. MILLER, H. I., and NASH, H. A., *Nature (London)* **290,** 523-526 (1981).
7. MILLER, H. I., and FRIEDMAN, D. I., *Cell* **20,** 711-719 (1980).
8. HOWE, M. M., *Virology* **54,** 93-101 (1973).
9. HOWE, M. M., O'DAY, K. J., and SCHULTZ, D. W., *Virology* **93,** 303-319 (1979).
10. FOWLER, R., DEGNEN, G., and COX, E., *Molec. Gen. Genet.* **133,** 179-191 (1974).
11. SCHUMM, J. W., MOORE, D. D., BLATTNER, F. R., and HOWE, M. M., *Virology* **105,** 185-195 (1980).
12. O'DAY, K. J., SCHULTZ, D. W., ERICSEN, W., RAWLUK, L. B., and HOWE, M. M., *Virology* **93,** 320-328 (1979).
13. WIJFFELMAN, C. A., WESTMAAS, G. C., and VAN DE PUTTE, P., *Virology* **54,** 125-134 (1973).
14. HOWE, M. M., SCHUMM, J. W., and TAYLOR, A. L., *Virology* **92,** 108-124 (1979).
15. WIJFFELMAN, C., and VAN DE PUTTE, P., *Molec. Gen. Genet.* **135,** 327-337 (1974).