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

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Received March 1, 1982; accepted March 22, 1982

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" inte 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 cts62 (8) by growth (9, slightly modified) in the mutD host KDI067 (10) and selected for Mu cts62 nuA mutants able to form plaques on lawns of a himA42 host (4) and Mu cts62 nuB mutants able to form large plaques on lawns of a himB114 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
The 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 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 nuA1 and nuA3 mutations can be rescued from prophage deletion strain MH165 (8) but not from strain KMBL1646 (13) whereas the wild type alleles of nuA106 and nuA107 cannot be rescued from either strain. The absence of nu" rescue from strain KMBL 1646, which carries a deletion ending between A1504 and A1093 indicates that the nuA alleles are located to the left of A1093; 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 DNA IN DELETED LYSOGENS

| STRAIN    | A | B | C | D | E | F | G | H | I | J | K | L | M | P | Q | V | W | R | U |
| MH132     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MH131     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MH130     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MH165     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| KMBL1646  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| KMBL1644  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MH166     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

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 A1504 and A1093 and the endpoint in KMBL1644 was located between B5150 and the pair B5175 and B5176. The rightmost deletion endpoint in strain MH165 was located to the left of the leftmost Aam 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 cts62 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°C. Individual am" plaques were resuspended in SM and spotted onto lawns of strains K37 (him") and K654 (himA422) (4) on TCMG plates which were scored for phage growth after overnight incubation at 37°C. 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.
alleles of all four nuB mutations were rescued from λMu 4M121-16 but not from λMu 4M145-1. λMu 4M121-16 is deleted for wild type alleles of all known G amber mutations but allows rescue of all I amber mutations; while λMu 4M145-1 is deleted for the DNA corresponding to all G amber mutations and the adjacent 6 I mutations (12).

Marker rescue of the nuB105 phage with hpMu 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 λMu 114-6 which carries even more Mu DNA than λMu 114-9 but still does not allow rescue of nu+, led us to disregard the single plaque observed with λMu 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 par-
ticles. Further experiments to define the
roles of himA and himI3 in Mu develop-
ment and the alterations present in the
nuA and nuB mutants are in progress.

ACKNOWLEDGMENTS

This work was supported by the College of Agri-
cultural and Life Sciences, University of Wisconsin,
Madison, Wisconsin, and by grants AI-12731 to
M.M.H. and AI-14363 to D.I.F. from the National In-
stitute of Allergy and Infectious Diseases and grant
NP-264 to M.M.H. from the American Cancer Society.
R.K.Y. was supported by training grant GM-07215
from the National Institute of General Medical Sci-
ences. M.M.H. is the recipient of Research Career
Development Award AI-00274 from the National In-
stitute of Allergy and Infectious Diseases.

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