The Use of Translocatable Genetic Elements to Construct a Fine-structure Map of the Klebsiella pneumoniae Nitrogen Fixation (nif) Gene Cluster

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The transposons Tn5, Tn7 and Tn10 and bacteriophage Mu have been used to derive insertion mutations in the Klebsiella pneumoniae nif gene cluster. A large number of deletion mutants have been derived by imprecise excision of insertion mutations and these deletions have been used to construct a fine-structure map of the nif cluster. Comparison of this genetic map with a physical map of the nif cluster derived by Reidel et al. (1979) showed a very good correlation between genetic and physical mapping methods.

A new complementation group, designated nifU, has been identified and mapped between nifN and nifS. Polarity studies on the 14 nif cistrons now identified suggests that they are organized in at least seven transcriptional units and that all the multicistronic units are transcribed in the same direction.

INTRODUCTION

Genetic analysis of nif mutations in Klebsiella pneumoniae has shown that all the genes specific to nitrogenase regulation and synthesis are located in a cluster near to the histidine operon. Fourteen nif cistrons arranged in seven transcriptional units have so far been identified (Merrick et al., 1978; Elmerich et al., 1978; MacNeil et al., 1978). Insertion mutations in the nif cluster have been derived using the transposons Tn5, Tn7 and Tn10 (Merrick et al., 1978) and bacteriophage Mu (MacNeil et al., 1978; Elmerich et al., 1978). These mutations have allowed analysis of the transcriptional organization of the nif genes and identification of some of the nif gene products (Roberts et al., 1978; Merrick et al., 1978; Elmerich et al., 1978).

Both the transposons Tn5, Tn7 and Tn10 and bacteriophage Mu can excise imprecisely from their point of insertion with the concomitant production of deletions (Kleckner et al., 1977; Toussaint et al., 1977; Merrick et al., 1978) and this property has allowed the isolation of a large number of deletion mutants with end-points in all the known nif cistrons. Using these deletions a fine-structure map of insertion mutants in the nif cluster has been constructed. The insertion mutants have also been the basis for a collaborative study of the physical distribution of genes in the nif gene cluster (Reidel et al., 1979) and the correlation observed between the genetic and physical maps indicates the advantages to be gained from combining these two techniques.
recombination analysis with a range of nifpoint mutations. In some cases the presence of an inversion was confirmed using P1 transduction (Kleckner et al., 1978). Chromosomal point mutations used.

donors for markers within the inverted segment but gave a nitrogen-free (NFDM) agar plate and the master plate was replicated on to this lawn. The resultant plate was incubated anaerobically for deletions as described below for the mapping of Mu insertions.

recipients.

grew in the mixed patch but not in unmated controls. The mapping of pCE1 nifdeletions was performed by some cistrons in a plate complementation test but could be demonstrated to have the relevant nifDNA by point mutations into the deletion strains. For these crosses the donor plasmid strains were patched on to nutrient agar (NA) plate to produce a master plate with up to 50 patches per plate which was incubated overnight at 37 °C. The following day 0.1 ml of an overnight culture of the recipient deletion strain was spread on histidine auxotrophs was in minimal medium supplemented with 1 

1978) was observed to be red-dependent and was therefore overcome by the use of chromosome as described by Merrick et al., 1978; Elmerich et al., 1978). Mu insertions (Muc+ or Mucfs) in plasmid pCE1 were isolated as described previously with phage Mphl and by mutagenesis of strain PC58 with phage Muhl. The isolation procedure was as described by Merrick et al., 1978) to obtain Mucfs insertions in plasmid pCE1.

Isolation of nif deletion and inversion strains. Plasmid deletions were selected as described by Elmerich et al., 1978). Chromosomal deletions were isolated following antibiotic enrichment for either drug-sensitive (Tn7, Tn10) or histidine-auxotrophic (Tn5, Tn7) derivatives of the chromosomal insertion mutants. The enrichment procedure was described by Merrick et al., 1978). Enrichment for histidine auxotrophs was in minimal medium supplemented with 1% histidine assay medium (Difco).

Complementation tests. Both qualitative and quantitative tests were carried out as described by Merrick et al., 1978). In most cases recA recipients were used. The problem of high-frequency homogenotization previously encountered when attempting to analyse nif::Tn5 mutations by complementation (Merrick et al., 1978) was observed to be recA-dependent and was therefore overcome by the use of recA derivatives of the chromosomal point mutations used.

Mapping the extent of deletions. Chromosomal deletions were mapped by crossing plasmids carrying nif point mutations into the deletion strains. For these crosses the donor plasmid strains were patched on to a nutrient agar (NA) plate to produce a master plate with up to 50 patches per plate which was incubated overnight at 37 °C. The following day 0-1 ml of an overnight culture of the recipient deletion strain was spread on a nitrogen-free (NFDM) agar plate and the master plate was replicated on to this lawn. The resultant plate was incubated anaerobically for 6 d at 30 °C. The exconjugants were scored as Nif+ when recombinants grew in the mixed patch but not in unmated controls. The mapping of pCE1 nif deletions was performed by scoring recombinants after introduction of the plasmids into a set of chromosomal point mutants and deletions as described below for the mapping of Mu insertions.

Mapping inversions. Strains carrying inversions were recognized by the fact that they failed to complement recA strains. The exconjugants were scored as Nif+ when recombinants grew in the mixed patch but not in unmated controls. The mapping of pCE1 nif deletions was performed by scoring recombinants after introduction of the plasmids into a set of chromosomal point mutants and deletions as described below for the mapping of Mu insertions.

METHODS

Bacteria, plasmids and bacteriophages. These are listed in Table 1 or have been described previously (Merrick et al., 1978; Elmerich et al., 1978; MacNeil et al., 1978). Strains with nif allele numbers 8200 to 8300 were derived by ethyl methanesulphonate mutagenesis.

Media and antibiotics. These were as described by Merrick et al., 1978) and Elmerich et al., 1978).

Construction of recA strains. This was performed as described by MacNeil et al., 1978) using a P1cm clv100 lysate grown on strain UN1290.

Isolation of insertion mutants. Transposon mutagenesis with Tn5, Tn7 and Tn10 has been described previously (Merrick et al., 1978). Mu insertions (Muc+ or Mucfs) in plasmid pCE1 were isolated as described by Elmerich et al., 1978). Klebsiella pneumoniae chromosomal insertion mutants were obtained by mutagenesis of strain UNF5023 with phage Mphl and by mutagenesis of strain PC58 with phage Muhl. The isolation procedure was as described by Elmerich et al., 1978) to obtain Mucfs insertions in plasmid pCE1.

Isolation of nif deletion and inversion strains. Plasmid deletions were selected as described by Elmerich et al., 1978). Chromosomal deletions were isolated after transduction of specific nif:::Tn mutations to the chromosome as described by Merrick et al., 1978). Deletions were isolated following antibiotic enrichment for either drug-sensitive (Tn7, Tn10) or histidine-auxotrophic (Tn5, Tn7) derivatives of the chromosomal insertion mutants. The enrichment procedure was described by Merrick et al., 1978). Enrichment for histidine auxotrophs was in minimal medium supplemented with 1% histidine assay medium (Difco).

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Mapping inversions. Strains carrying inversions were recognized by the fact that they failed to complement some cistrons in a plate complementation test but could be demonstrated to have the relevant nifDNA by recombination analysis with a range of nif point mutations. In some cases the presence of an inversion was confirmed using P1 transduction (Kleckner et al., 1979). In reciprocal crosses such strains acted as good donors for markers within the inverted segment but gave low numbers of recombinants when used as recipients.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype or phenotype</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNF107</td>
<td>rpsL1 Δ107 (gnd his nif)</td>
<td>Dixon et al. (1977)</td>
</tr>
<tr>
<td>UN1290</td>
<td>hisD4226 recA56 srl-300::Tn10</td>
<td>MacNeil et al. (1978)</td>
</tr>
<tr>
<td>UN1990</td>
<td>nifV4944</td>
<td>MacNeil et al. (1978)</td>
</tr>
<tr>
<td>PC58</td>
<td>hisD2 hsdR1 rpsL4 Mu-sensitive</td>
<td>This work</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRD1</td>
<td>Km Te Cb Gnd His Nif ShiA Tra IncP</td>
<td>Dixon et al. (1976)</td>
</tr>
<tr>
<td>pMF100</td>
<td>Gnd His Nif ShiA Tra</td>
<td>M. Filser &amp; F. Cannon</td>
</tr>
<tr>
<td>pCE1</td>
<td>Mu-sensitive derivative of pRD1</td>
<td>Elmerich et al. (1978)</td>
</tr>
<tr>
<td>Phages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muc+</td>
<td>Mu phage with P1 host range</td>
<td>A. Toussaint</td>
</tr>
<tr>
<td>Mucfs 62</td>
<td>Mu phage with modified host range; capable of propagation on some Klebsiella strains</td>
<td>A. Toussaint</td>
</tr>
<tr>
<td>Mphl</td>
<td>Mu phage with modified host range; capable of propagation on some Klebsiella strains</td>
<td>This work</td>
</tr>
</tbody>
</table>

Table 1. Bacteria, plasmids and bacteriophages
**Results**

As different cistronic nomenclatures were used by Merrick *et al.* (1978) and MacNeil *et al.* (1978) a standard nomenclature for the nif gene cluster has been agreed and will be used for the remainder of this report and in all future publications. The original designations and the new standard designations are given in Fig. 1.

**Isolation of deletion strains**

Tetracycline-sensitive derivatives of Tn10 insertions either retained their original Nif- phenotype or were deletions or inversions with one end-point apparently at the original insertion site. In no case did tetracycline-sensitive derivatives give a Nif+ phenotype suggesting that precise excision of the transposon was rare.

With Tn5 and Tn7, selection for histidine auxotrophy allowed independent screening for deletion or inversion formation and for loss or retention of the transposons. A variety of excision events were observed including three classes of deletion: class I, with an end-point at the original insertion site; class II, deletions extending through the original insertion site; class III, where the deletion did not extend as far as the insertion site. Examples of the three classes of excision event are given in Fig. 2. All three classes could be obtained from a single insertion mutation. In class I and class II derivatives the antibiotic resistance markers of the transposon were often retained but no attempt was made to determine whether the transposon was still nif-linked or whether it had been relocated on the chromosome. Whenever possible, deletions which had lost the drug resistance phenotype of the transposon were used

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Merrick *et al.* (1978)  
Elmerich *et al.* (1978)  
MacNeil *et al.* (1978)  
Standard nomenclature

|------------------------|--------------------------|-------------------------|-----------------------|

Fig. 1. Standard nomenclature for *K. pneumoniae* nif cistrons.
Plasmid nif mutation

| Parent UNF1142 nifE2182::Tn7 | His | B | A | F | M | V | S | U | N | E | K | D | H | J | Tp |
|-----------------------------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + + + + + + + + rec | rec | + + + + | R |
| Class I | - - - - - - - - - - rec | + + + + | R or S |
| Class II | - - - - + + + + rec | + + + | R or S |
| Class III | - - - + + + + | R |

Fig. 2. Examples of the three classes of deletion obtained from Tn7 or Tn5 insertions. The parent strain UNF1142 and his derivatives were tested in a plate complementation assay against plasmid-borne point mutants for each nif cistron: +, complementation; -, no complementation; rec, some Nif+ recombinants. All strains were scored for trimethoprim (Tp) resistance (R) or sensitivity (S).

for mapping purposes in preference to deletions which had retained the phenotype. Class III derivatives always retained the drug resistance markers of the transposon.

Three categories of heat-resistant survivors were obtained from strains carrying Mucts plasmid insertions: (1) mutations with partial nif deletions with an end-point at the original insertion site (analogous to class I above), these strains were either Mu-immune or Mu-sensitive; (2) mutants with total or partial nif deletions extending through the original insertion site (analogous to class II), these were all Mu-sensitive and, where examined, total nif deletions had lost 50 to 70% of the plasmid DNA; (3) mutants with no nif deletion which were apparently impaired in Mu-killing functions and were all Mu-immune.

Reversion analysis

Nif::Tn5 mutants reverted to Nif+ at a frequency of $10^{-6}$ to $10^{-2}$ and all revertants tested retained kanamycin resistance. Spontaneous kanamycin-sensitive derivatives of Tn5 occurred at a frequency of less than $5 \times 10^{-4}$ and it is not possible to enrich for kanamycin-sensitive derivatives.

Only 50% of the nif::Tn7 mutants tested reverted to Nif+ and the reversion frequency was $10^{-8}$ to $10^{-9}$. Again all revertants retained the antibiotic resistance markers of the transposon. Precise excision of Tn7 was never observed following selection for trimethoprim sensitivity.

Not all Tn10 mutants reverted to Nif+ but of those which did the reversion frequency varied from $10^{-6}$ to $10^{-9}$. The majority of Nif+ revertants retained tetracycline resistance. Hence, with all three transposons (Tn5, Tn7 and Tn10), when precise excision was selected by reversion of the nif::Tn mutation to Nif+, nearly all revertants retained the antibiotic resistance markers of the element but no attempt was made to determine the location of the transposon in these revertants.

Mutations caused by the insertion of wild-type Mu do not revert, at least in Escherichia coli (Taylor, 1963). Several Muc+ insertions in each cistron were tested for reversion. Nif+ revertants were obtained only in the case of nifA8502::Muc+, with a frequency of about $10^{-8}$. A proportion of the survivors from Mucts insertions should carry MuX prophages (Bukhari, 1975) which are known to revert. However, no Nif+ revertants were obtained from those survivors tested.

Mapping

Insertion mutations in the plasmids pRD1, pMF100 and pCE1 were mapped against a large number of chromosomal deletion strains as described in Methods. When an insertion was located within a deletion interval covering two adjacent cistrons assignment of the insertion to a specific cistron was based on complementation analysis. A total of 397 insertion mutations were mapped into 84 deletion intervals in the nif gene cluster (Figs 3 and 4).
Fig. 3. Map of Mu-induced nif deletions in plasmid pCE1. Deletions were mapped against point and insertion mutations in the K. pneumoniae chromosome. Allele numbers for point and insertion mutations are listed above each gene and for deletions are given at the end-point of the deletion.
Fig. 4. Map of nif deletions in the K. pneumoniae chromosome. Deletions were mapped against point and insertion mutations in plasmids pRD1, pMF100 and pCE1 and allele numbers for these mutations are listed above each gene. Allele numbers for deletions are given at the end-point of the deletion.

To facilitate comparison with the physical map of Reidel et al. (1979) the following should be noted: for Mu insertions 8000 should be added to the figures given by Reidel et al. (1979; Fig. 5) to derive relevant allele numbers; for Tn5 insertions 2300 should be added to pMF numbers to derive allele numbers; for Tn10 insertions pMF125 is nifB2018, pMF106 is nifA2013, pMF103 and

All nif insertion mutants reported in Figs 3 and 4 gave rise to Nif+ recombinants when introduced into recipients carrying a point mutation in the gene in which the insertion was located. We therefore assumed that extensive deletions were not created upon insertion.

Tn10 insertions in the nif gene cluster were significantly non-random, with the majority of insertions occurring in two deletion intervals within nifJ. Tn5, Tn7 and Mu insertions appeared to be random and insertions were obtained in 13 cistrons. No insertions were obtained in nifQ and this was probably due to the leaky nature of the mutations or a problem of definition (see later).

The plasmid pRD209 was found to carry a mutation nif-2209::Tn7 mapping in nifU and
Fine-structure map of Klebsiella nif genes

UNF2038 are nifM2011, pMF104 and UNF2040 are nifS2012, pMF113 and UNF143 are nifN2014, pMF114 is nifE2027, pMF124 is nifK2038, pMF107 is nifJ2016, pMF102 and UNF2027 are nifJ2009.

not in nifS as previously reported. Nif-2184::Tn7 was confirmed as being in nifE as suggested by complementation data (Merrick et al., 1978). The plasmid pLS23, previously reported as a nifE::Mu insertion on the basis of complementation data, was reassigned as nifS8023::Muc+.

Complementation analysis and polarity

Eleven complementation groups \([NifB, nifA, nifF, nifM, nifS (previously nifN), nifN (previously nifI), nifE, nifK, nifD, nifH and nifJ]\) were defined by Merrick et al. (1978). A further two cistrons nifQ and nifV were described by MacNeil et al. (1978). We have now
Table 2. Complementation analysis of nifU2461

Results are normalized to a percentage of the activity given by the Nif+ plasmid (pRD1, pMF100 or pCE1) in each mutant background.

<table>
<thead>
<tr>
<th>Recipient allele</th>
<th>Plasmid donor allele</th>
<th>A107 (gnd his nif)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nifB4106</td>
<td>nifA2263</td>
</tr>
<tr>
<td>None</td>
<td>0.02</td>
<td>3.5</td>
</tr>
<tr>
<td>Nif+</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>nifU2461</td>
<td>82</td>
<td>72</td>
</tr>
</tbody>
</table>

Table 3. Complementation analysis of insertion mutations in the nifUSVM gene cluster

Results are normalized to a percentage of the activity given by the Nif+ plasmid (pRD1, pMF100 or pCE1) in each mutant background.

Data for nif::Tn5 mutations are from complementation analysis with recA derivatives of the recipient mutants listed.

<table>
<thead>
<tr>
<th>Recipient allele</th>
<th>Plasmid donor allele</th>
<th>A107 (gnd his nif)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nifF4066</td>
<td>nifM2104</td>
</tr>
<tr>
<td>None</td>
<td>0.02</td>
<td>0.2</td>
</tr>
<tr>
<td>Nif+</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>nifM8046::Muc+</td>
<td>0.7</td>
<td>47</td>
</tr>
<tr>
<td>nifM2577::Tn5</td>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td>nifM2662::Tn7</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>nifV2429::Tn7</td>
<td>46</td>
<td>27</td>
</tr>
<tr>
<td>nifS8031::Muc+</td>
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<td>nifS2012::Tn5</td>
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<td>15</td>
</tr>
<tr>
<td>nifS2195::Tn5</td>
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</tr>
<tr>
<td>nifU8116::Mucts</td>
<td>3.3</td>
<td>95</td>
</tr>
<tr>
<td>nifU2562::Tn5</td>
<td>0.7</td>
<td>25</td>
</tr>
<tr>
<td>nifU2209::Tn7</td>
<td>5</td>
<td>55</td>
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</table>
recognized a new complementation group defined by the point mutation nif-2461 and designated nifU (Table 2). NifU2461 was 38% cotransducible with hisD2 and mapping data indicated a location between nifS and nifN (Fig. 4). Six nifU::Tn and ten nifU::Mu insertion mutations have been isolated. All these insertions were strongly polar on nifS (Table 3). No polarity of nifU insertions on nifN or nifE or of nifN insertions on nifU was observed and hence we conclude that nifU and nifS belong to a single transcriptional unit transcribed from nifU to nifS.

The effect of either nifU or nifS insertions on both nifV and nifM is very difficult to ascertain. All complementation studies with nifV are complicated by the leakiness of the nifV mutations (see above) and no clear polar effects of nifU or nifS insertions can be observed. No polarity of nifU or nifS insertions on nifM is observable by acetylene reduction (Merrick et al., 1978; and Table 3), but some effect is apparent by plate complementation. The same situation applies to deletion nif-2472 starting outside nifI and ending in nifS which has no significant effect on nifM activity in assays but some effect in plate complementation.

Some effect of nifM::Tn5 insertions on nifF complementation has consistently been observed in plate complementation but not by acetylene reduction (Merrick et al., 1978). This effect was confirmed with all nifM::Tn5 mutants reported here but no such effect was observed with other insertions in nifM.

No unambiguous nifQ mutants were identified in this study but three insertion mutants (nif-2739::Tn7, nif-2740::Tn7 and nif-2574::Tn5) were isolated which are nifB in complementation and are leaky in acetylene reduction. The two Tn7 insertions map within the nif-4303 deletion of MacNeil et al. (1978) and are therefore within nifQ as defined by MacNeil et al. (1978). Leaky Mu-induced nifB mutations were also described by MacNeil et al. (1978) and hence it is not clear to us whether nifQ is definitely an independent cistron.

NifV was described by MacNeil et al. (1978) and it was reported that all non-polar nifV point mutations are very leaky. One such strain UN1990 (nifV4944) had 45% wild-type activity when assayed by us for acetylene reduction and grew quite well anaerobically on nitrogen-free medium. The allocation of mutations to the nifV cistron was therefore impossible by acetylene reduction assays and extremely difficult by assessment of growth on nitrogen-free medium. Assignment of mutations to nifV was based on plate complementation, leaky phenotype and map position. NifV::Tn7 mutations had activities of 50 to 100% by acetylene reduction even though such mutations should completely inactivate the nifV gene product.

Using complementation analysis, polarity studies have been carried out with all the insertion mutations described here. The results suggest that the nif gene cluster contains seven or eight transcriptional units:

nifJ, nifHDK, nifEN, nifUS(V), nifM, nifF, nifA/L, nifB(Q)

This is essentially in agreement with previous reports (Elmerich et al., 1978; MacNeil et al., 1978; Merrick et al., 1978), with the exception of the nifUSVM cluster which may contain two transcripts (see Discussion).

It is not yet clear whether nifA/L comprises one or two genes. MacNeil et al. (1978) suggest that nifL [originally designated as a separate gene by Kennedy (1977) on the basis of transductional mapping] is not required for growth on nitrogen and hence mutations in nifL are only detected if they are polar on nifA. There is therefore no precise phenotype for all nifL mutations and we have not attempted to define a boundary between nifA and nifL (Figs 3 and 4). The ability of some Mu insertions to give NiF revertants was the property used by MacNeil et al. (1978) to divide the nifA complementation group into two genes. Mutations in the five his-distal deletion intervals of the nifA region were assigned to nifL on the basis that the majority of Mu insertions in this region reverted to NiF+. A similar distinction could not be made with Tn5, Tn7 and Tn10 mutations as these transposons
can excise precisely. Reversion to Nif+ was observed with one Mu insertion nifA8502::Muc+ isolated in this study and located in the his-distal part of the nifA/L region.

DISCUSSION

Virtually all studies of transposition and related phenomena to date have been carried out in Escherichia coli and/or Salmonella typhimurium, but there appear to be some differences in behaviour of the insertion elements used in this study when examined in Klebsiella pneumoniae.

The site specificities of integration into the nif gene cluster are similar to those reported for other genetic systems in E. coli using Tn5 (Berg, 1977), Tn10 (Kleckner et al., 1977) and Mu (Bukhari & Zipser, 1972). Tn7 has been reported to integrate preferentially at one site in the E. coli chromosome (Barth et al., 1976) but, in contrast, no significant specificity of Tn7 integration into nif was observed. Furthermore, the transposition of Tn7 into RP4 has been reported to cause frequent deletions of up to 2 megadaltons at the point of insertion (Barth et al., 1978). No such deletions were detected when Tn7 was inserted into the nif gene cluster.

The characteristics of deletions and inversions formed by Tn10 in K. pneumoniae appeared to be similar to those described in S. typhimurium (Kleckner et al., 1977) although the deletion end-points may be more random than observed in Salmonella (Dale Noel & Ferro-Luzzi Ames, 1978). The generation of deletions with one end-point always at the original insertion site is also observed with Tn3 (Nisen et al., 1977).

In contrast, Mu produces two classes of deletions in both E. coli and K. pneumoniae (Toussaint et al., 1977; and this paper): deletions with one end-point at the insertion site (class I) and deletions extending to both sides of the insertion site (class II). Deletion formation by Tn5 and Tn7 has not previously been reported, but in the nif gene cluster these transposons can produce three classes of deletion: class I and class II, as described above for Mu, and class III deletions where the transposon is always retained but the deletion is not continuous with the original site of the insertion. In class III deletions we did not analyse whether or not the DNA between the original insertion site and the deletion end-point was inverted. If an inversion is involved in the generation of class III strains the transposon could be located at either end of the inverted segment. Such strains are probably not stable and can apparently become deleted for the inverted segment (M. J. Merrick & M. Filser, unpublished observations).

The reversion frequencies of nif mutations generated by Tn5, Tn7 and Tn10 are similar to those quoted for other genes in E. coli and S. typhimurium, i.e. lacZ::Tn5 (Berg, 1977), tra::Tn7 (Barth & Grinter, 1977), hisG::Tn10 (Kleckner et al., 1979) and lacZ::Tn10 (Foster, 1977). However, with Tn5 and Tn10 in E. coli and S. typhimurium the majority of revertants are drug-sensitive, presumably as a result of precise excision and loss of the transposon. Around 1% of Tn5 insertions in E. coli lac are kanamycin-resistant and it is suggested that in these revertants the transposon was re-inserted in a new site prior to the reversion event. By comparison, all Nif+ revertants obtained in K. pneumoniae from nif::Tn5 and nif::Tn7 mutations and most revertants from nif::Tn10 mutations retained the transposon. In the majority of cases the phenotype of the nif::Tn mutation precludes reversion by a suppressor mutation since most nif genes are believed to code for structural proteins and Mu insertions in all nif genes except nifL cannot be suppressed. Therefore, these revertants must either have arisen in a clone in which transposition had occurred prior to the reversion event, or reversion must be linked to transposition. These differences in precise excision of the transposon between E. coli and K. pneumoniae may reflect differences in host proteins involved in the transposition process.

Whilst the polar effects of the insertion mutations described here suggest a transcriptional
organization which is essentially in agreement with previous reports (Merrick et al., 1978; Elmerich et al., 1978; MacNeil et al., 1978), the precise transcriptional organization of the central cluster nifUSVM is difficult to determine. The absence of any strong polar effect of nifU and nifS insertions on nifM may indicate that either there is a secondary promotor between nifS and nifM which can allow transcription initiation after nifS or that nifUS and nifM represent separate transcriptional units. Both possibilities are supported by the complementation phenotypes of deletions extending into nifS or nifV from the right-hand side of the nif cluster. Such deletions, which have been described in this paper and which were also described by MacNeil et al. (1978), complement nifM mutants even though the nifUS promotor is deleted. MacNeil et al. (1978) suggest that this result is due to the fusion of nifM to other promoters within or outside the nif cluster. However, the majority of their deletions extend outside nif and would therefore have to be fused either to constitutive promotores or to promoters under similar control to the nifUS promotor.

The insertion of a transposon into a nif gene increases the size of the EcoRI restriction fragment on which the gene is located by an amount equal to the size of the transposon. This increase can be exploited in conjunction with suitable radioactive probes derived from amplifiable plasmids carrying cloned nif DNA fragments (Cannon et al., 1979) to determine the distribution of the nif genes on EcoRI restriction fragments. Using this approach 85 nif insertion mutations, whose genetic mapping and complementation analysis is described in this paper, have also been assigned physical locations in the K. pneumoniae nif gene cluster (Reidel et al., 1979).

The availability of both a genetic and a physical map of a large number of insertion mutations in the nif gene cluster allows comparison to be made between the two mapping methods. The physical map was constructed quite independently of the genetic map reported here and is virtually identical to the genetic map in the relative allocations of the insertion mutants. However, when making comparisons between the two maps the following discrepancies should be noted. The plasmids pMF320 and pMF321 carry Tn5 insertions in nifN (nifN2620 and nifN2621, respectively) and not in nifE as indicated by Reidel et al. (1979). Plasmids pMF308 and pMF318 were inadvertently interchanged on the physical map and hence pMF308 and pMF318 refer to nifJ2618 and nifJ2608 on the genetic map.

Some of the Tn10 strains mapped by Reidel et al. (1979) carried the same nif: Tn10 allele either on the plasmid or on the chromosome; these are UNF2038 and pMF103 both carrying nifM2011: Tn10, UNF2040 and pMF104 both carrying nifS2012: Tn10, UNF143 and pMF113 both carrying nifN2014: Tn10 and UNF2017 and pMF102 both carrying nifJ2009: Tn10. In the case of nifM2011: Tn10 the orientation of the transposon was apparently reversed as a result of transduction from the plasmid to the chromosome. In all these cases the two insertions were mapped to within 0-2 kilobase of each other (as estimated from the physical map) and hence if the location of the transposon was unchanged by transduction from the plasmid to the chromosome this is an indication of the sensitivity of the physical mapping technique.

When the above points are taken into consideration it is clear that the agreement between the genetic and physical maps of the nif cluster is extremely good. Whilst these two methods are not interchangeable they are clearly complementary. For example, if one wishes to clone small fragments of the nif cluster, such as particular nif promotores, suitable mutants can be chosen which will be within the required fragment, thus allowing selection of the required clones by marker rescue.

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