NOTES

Physical and Genetic Characterization of Deletions in *Streptococcus pneumoniae*

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Genetic properties of markers may discriminate between deletions and point mutations. We have designed a physical method for a direct characterization of deletions which also gives an estimate of their size.

Besides the usual criterion of differential reversion rates, mutations in pneumococci exhibit characteristic differences in efficiency of transformation. Single-site markers fall into at least three classes which do not overlap: low, high, and very high efficiency of transformation (4, 7, 14). Unlike point mutations, integration efficiencies of multisite markers corresponding to deletion mutants vary over a wide continuous distribution (7). The rate of transformation of deletion mutations is not affected by the *hex* function, which discriminates between point mutations (8). Recently, in a study on the efficiency of the individual strands, using artificial heteroduplex DNAs, we found that both strands of a multisite marker (*amiA30*) which exhibits a high efficiency of transformation are equally active in transformation, whereas there is a strand preference for high-efficiency point mutations (3). Since two other markers (*amiA28* and *amiA109*) behave similarly to *amiA30* with respect to strand preference and *hex* action, it has been proposed that they are also multisite markers (3). The experiments described here were designed to further characterize the three markers as deletions, using genetic properties which discriminate deletions and point mutations and a physical method which also gives an estimate of their size.

The *amiA30* mutation does not recombine with two distinct sites, *amiA5* and *amiA17* (4) (see Fig. 1); moreover, this mutation removes an *Eco*RI site in the *amiA* locus (1). These observations led us to suggest that the mutation is a deletion. Similarly, *amiA109* does not recombine with sites *amiA9*, *-20*, and *-24* and could extend appreciably to the left of *amiA9* and to the right of *amiA24* (see Fig. 1). Because similar attempts to find sites covered by the *amiA28* marker were unsuccessful, we have looked for another prop-

erty of multisite markers. It has been reported for the *amiA30* marker that UV sensitivity is much higher for a DNA carrying the wild-type allele than for one carrying the mutant allele (4). A similar result was obtained with a relatively small multisite marker of the amylomaltase locus (6). The hypersensitivity of the wild-type allele could be accounted for by its physical size; i.e., it behaves as a multiple target to UV irradiation. To determine whether the *amiA28* marker meets this criterion for multisite mutations, DNAs from *amiA⁺ str-41* or *amiA28 str-41* strains were UV irradiated and used to transform strains *amiA28* and wild type, respectively. Inactivation curves of the two DNAs show that the wild-type allele for the *amiA28* marker is 3.5-fold more UV sensitive than its aminopterin-resistant counterpart (Fig. 2). The similar behavior of *amiA28* and *amiA30* markers suggests that *amiA28* also could be a multisite marker.

Thus, according to genetic criteria, markers *amiA30*, *-28*, and *-109* could be deletions. We have used these markers for physical studies on their nature. Restriction enzymes cut DNA at specific sites so that genetic markers are carried by well-defined fragments of DNA. These fragments can be separated by agarose gel electrophoresis, and those carrying specific markers can be detected by their transforming activity after recovery from the gels (1, 5). Since the mobility of a DNA fragment is essentially correlated to its size, a fragment carrying a deletion will exhibit a higher mobility than the corresponding wild-type fragment. Therefore, the size of a deletion could be inferred from this difference in mobility of restriction fragments.

Two *Eco*RI-generated fragments corresponding to the *amiA* locus have been found (1). Marker *amiA109* is located within the A fragment (Fig. 1). DNAs from the wild-type strain
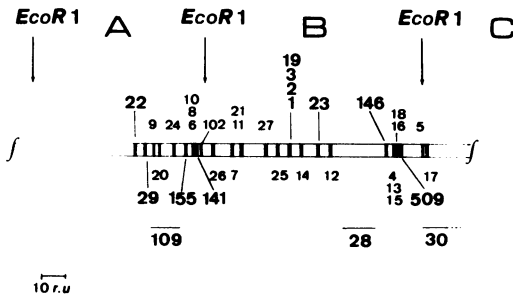
FIG. 1. Map of the amiA locus. Mutations of the amiA locus confer a resistance to 10^{-5} M aminopterin (10). These mutations are ordered according to their map position established from genetic crosses (11). The bar represents 10 recombination units. Recombination units are defined as percentage of wild-type recombinant frequency corrected for the efficiency of the recipient strain. EcoRI sites are indicated by arrows. Correlation between genetic and physical distances as well as location of EcoRI sites have been described (1). Molecular weights of EcoRI-generated fragments A and B are 1.20×10^6 and 1.66×10^6, respectively.
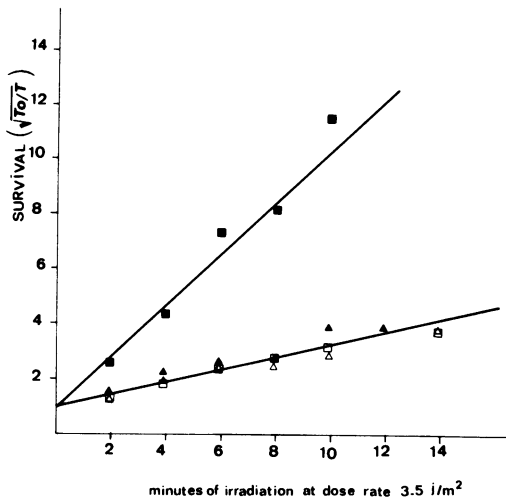

FIG. 2. Relative rates of inactivation of wild-type and mutant alleles of amiA28 by UV irradiation. Two DNAs, each carrying the reference marker str-41 and either amiA28 or its wild-type counterpart, were UV irradiated as described by Ephrussi-Taylor et al. (4). Residual transforming activity of both amiA28 and amiA$^+$ markers and of the reference marker str-41 were titrated on wild-type and amiA28 mutant recipients. Transformation and selection of am28 and am$^+$ markers were as described previously (10, 13). The inverse square of survival, $\sqrt{T0/T}$, is plotted versus UV dose, according to the analysis of Rupert and Goodgal (9). T0 is the initial number of transformants, and T is the number of transformants after UV irradiation. Symbols: ($\triangle$) str41-; ($\square$) amiA28; ($\blacktriangle$) str41-; ($\blacksquare$) amiA$^+$.

or a strain bearing the amiA109 marker were treated by EcoRI, mixed, and run into a 0.6% agarose gel (see legend to Fig. 3). DNA was recovered by squeezing frozen, 2-mm-wide gel slices (12) and assayed for transforming activity on the amiA109 and wild-type recipient strains. The position of the A$^+$ fragment will be defined by the yield of wild-type transformants, using amiA109 as recipient strain. Similarly, the position of the A fragment carrying the amiA109 marker will be determined by the aminopterin-resistant transformants obtained with the wild-type strain as recipient (Fig. 3). The A$^+$ fragment
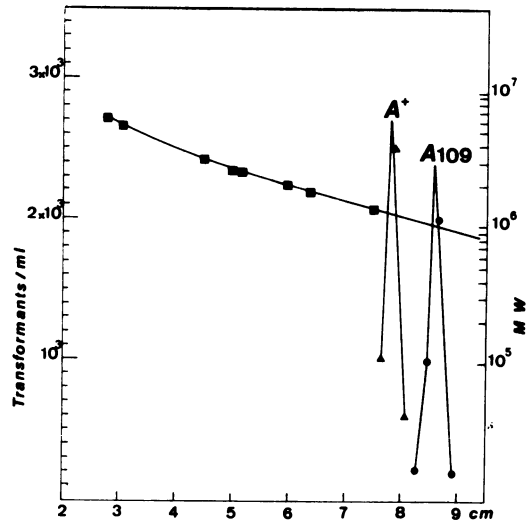

FIG. 3. Physical characterization of the amiA109 mutation. The electrophoretic mobility of the EcoRI-generated A fragment bearing the amiA109 mutation has been compared with that of the wild-type A fragment. Conditions for EcoRI digestion and horizontal agarose gel electrophoresis have been described (1). DNA was recovered from gel by freeze-squeezing 2-mm-wide gel slices (12), diluted with transformation medium, and assayed for transforming activity. Two samples were run within the same gel: (i) a mixture of EcoRI-cleaved DNA from strain amiA109 (5 µg) and wild type (5 µg); (ii) HpaI-cleaved λ CI857S7 DNA (0.5 µg). Gel from (i) was sliced into 2-mm pieces and assayed for transforming activity. Gel from (ii) was stained in ethidium bromide solution (2 µg/ml). Transforming activity to aminopterin of the A fragment bearing amiA109 was assayed on the wild-type recipient ($\bullet$). Transforming activity of the A$^+$ fragment was assayed on the amiA109 recipient ($\blacktriangle$). The curve of molecular weight (MW) versus mobility of λ fragments produced with endonuclease HpaI has been established from (ii). ($\blacksquare$) Molecular weights of fragments A and A109 are inferred from their mobilities measured at maximum transforming activity and are, respectively, 1.20×10^6 and 0.95×10^6.

exhibits a slower electrophoretic mobility than the A *amiA109* fragment. Thus, *amiA109* is a deletion. Its size can be estimated by comparison of the sizes of A^+ (1.2 megadaltons) and A *amiA109* (0.95 megadalton) fragments evaluated from their mobilities, using as size standards *Hpa*I-generated λ fragments. The size of the *amiA109* deletion would be 0.25 megadalton or 380 base pairs. In similar experiments not shown, we found that the B *amiA28* fragment is shorter than the B^+ fragment by an estimated 240 base pairs. It has been possible to confirm that *amiA30* is a deletion by a similar experiment with a slight modification. Since this mutation removes an *Eco*RI site, we have compared the electrophoretic mobilities of either the fragment carrying it or the B+C fragment resulting from a partial hydrolysis of the wild-type DNA. This B+C fragment is defined by its ability to transform the *amiA30* recipient to wild type. The estimated size of the *amiA30* deletion would be 200 base pairs. However, this estimation is less accurate than for *amiA28* and *amiA109* since for technical reasons wild-type and mutant DNAs have been run into separate slots. Moreover, since larger DNA fragments are involved, the resolution power of the gel is reduced.

It is noteworthy that the relationship between genetic distances and physical length established for large distances appears to be verified also for short distances. For example, the *amiA109* deletion extends over a distance of 12 recombination units, which would give an estimate of 360 base pairs using the relationship 1 recombination unit for 30 nucleotides (1). The physical size found for *amiA109* (380 base pairs) is in good agreement with this expected value.

The suggestion on genetic grounds that the three markers studied are deletions is corroborated by the more direct physical evidence that we have presented. The lengths of these deletions have been estimated. It appears that genetic criteria such as absence of strand preference for high-efficiency markers or differential UV inactivation rates between wild-type and mutant alleles are good indications of the deletion nature of a mutation. Another genetic criterion can be used to characterize deletions: the dramatic increase in recombination rates of neighboring markers when a deletion is carried on donor DNA (2).

The method for physical characterization of deletions can be used only for deletions long enough to give a differential mobility on electrophoresis. The lower limit can be estimated to a few tens of nucleotides. This method has been useful for characterizing deletions induced by transformation with cloned pneumococcal DNA (2) and also could be applied to characterization of DNA additions.

LITERATURE CITED

1. Claverys, J. P., H. Lataste, and A. M. Sicard. 1979. Localization of two *Eco*RI restriction sites within the *amiA* locus in pneumococcus. Relationship between the physical and the genetic map, p. 131–169. *In* S. W. Glover and L. O. Butler (ed.), Transformation 1978. Cotswold Press Ltd., Oxford.
2. Claverys, J. P., J. C. Lefèvre, and A. M. Sicard. 1980. Transformation of *Streptococcus pneumoniae* with *S. pneumoniae*-Lambda phage hybrid DNA. Induction of deletions. Proc. Natl. Acad. Sci. U.S.A. 77:3534–3538.
3. Claverys, J. P., M. Roger, and A. M. Sicard. 1980. Excision and repair of mismatched base pairs in transformation of *Streptococcus pneumoniae*. Mol. Gen. Genet. 178:191–201.
4. Ephrussi-Taylor, H., A. M. Sicard, and R. Kamen. 1965. Genetic recombination in DNA-induced transformation of pneumococcus. I. The problem of relative efficiency of transforming factors. Genetics 51:455–475.
5. Harris-Warrick, R. M., Y. Elkana, S. D. Erlich, and J. Lederberg. 1975. Electrophoretic separation of *Bacillus subtilis* genes. Proc. Natl. Acad. Sci. U.S.A. 72:2207–2211.
6. Lacks, S. 1965. Genetic recombination in pneumococcus, p. 159–164. *In* M. Kohoutová and J. Hubáček (ed.), The physiology of gene and mutation expression. Proceedings of the Symposium on the Mutation Process. Academia, Prague.
7. Lacks, S. 1966. Integration efficiency and genetic recombination in pneumococcal transformation. Genetics 53:207–235.
8. Lacks, S. 1970. Mutants of *Diplococcus pneumoniae* that lack deoxyribonucleases and other activities possibly pertinent to genetic transformation. J. Bacteriol. 101:373–383.
9. Rupert, C. S., and S. H. Goodgal. 1960. Shape of ultraviolet inactivation curves of transforming deoxyribonucleic acid. Nature (London) 185:556–557.
10. Sicard, A. M. 1964. A new synthetic medium for *Diplococcus pneumoniae* and its use for the study of reciprocal transformation at the *amiA* locus. Genetics 50:31–44.
11. Sicard, A. M., and H. Ephrussi-Taylor. 1965. Genetic recombination in DNA-induced transformation of pneumococcus. II. Mapping the *amiA* region. Genetics 52:1207–1227.
12. Thuring, R. W. J., J. P. M. Sanders, and P. Borst. 1975. A freeze squeeze method for recovering long DNA from agarose gels. Anal. Biochem. 66:213–220.
13. Tiraby, G., and M. S. Fox. 1974. Marker discrimination and mutagen induced alteration in pneumococcal transformation. Genetics 77:449–458.
14. Tiraby, G., and M. A. Sicard. 1973. Integration efficiencies of spontaneous mutant alleles of *amiA* locus in pneumococcal transformation. J. Bacteriol. 116:1130–1135.