TRANSMISSION OF GENETIC INFORMATION DURING TRANSFORMATION IN *Diplococcus pneumoniae*

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Received February 17, 1968

It is generally accepted that genetic transformation in pneumococcus requires the physical insertion of a single stranded fragment of DNA into the genome of a recipient organism (Lacks, 1962 ; Guild & Robinson, 1963 ; Fox & Allen, 1964). In pneumococcal transformation, markers are characterized by their efficiency (Sicard, 1964 ; Ephrussi-Taylor, Sicard & Kamen, 1965 ; Lacks, 1966). There occur high (H.E.) and low (L.E.) efficiency markers, the behavior of which has been already widely studied (Lacks, 1966 ; Ephrussi-Taylor, 1966 ; Sicard & Ephrussi-Taylor, 1965 and 1966). Guild & Robinson (1963) suggested that the genetic information of an H.E. marker remains in only one of the two strands of the recipient DNA until the first chromosomal replication. More recently Ephrussi-Taylor (1966) presented the hypothesis that L.E. markers modify the two strands of the recipient DNA.

We have compared the behavior of H.E. and L.E. markers during transformation by a new method involving only pure genetical experiments. Our results support the hypothesis that the genetic information of H.E. markers is transmitted mainly into a single strand of the recipient DNA whereas it is transmitted into both strands for the L.E. markers.

MATERIALS AND METHODS

a) Principle :

The method consists of an analysis of the progeny of the bacteria transformed by a DNA bearing two independent genetical markers. The

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colonies of bacteria transformed for one character (marker A) are isolated, and the isolates analysed for the presence of the second character (marker B). Those colonies which represent the progeny of doubly transformed bacteria are selected and their composition studied.

If, in the original transformed cell, both strands of the recipient molecule are modified identically by both markers, the progeny will be pure, i.e. all the cells of such a colony will possess both markers. Conversely, if both strands are modified by the first selected character while only one is modified by the second selected marker, the progeny will be mixed, i.e. part of the population would remain untransformed for the second selected marker (Figure 1).

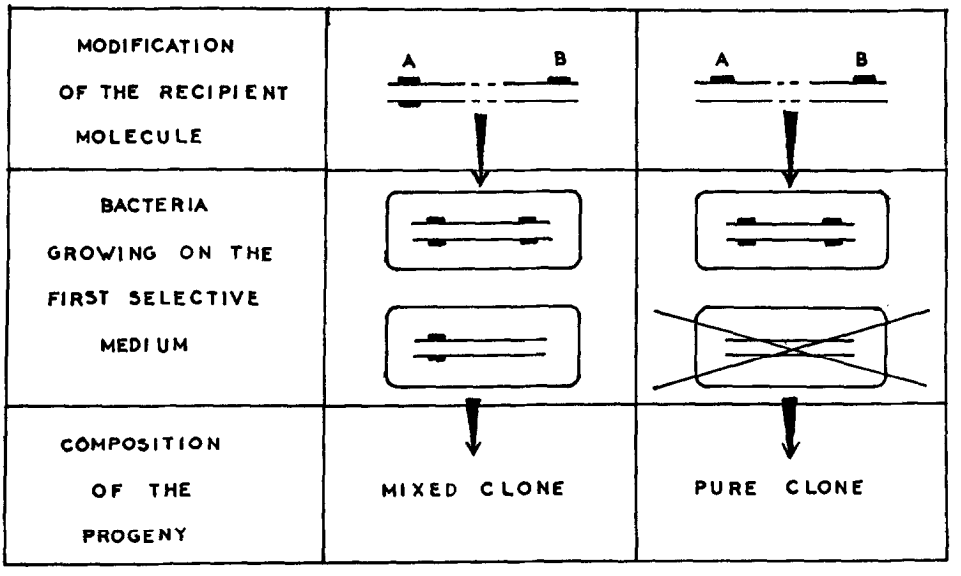

Figure 1 : Progeny of doubly transformed bacteria after a first selection for marker A.

The frequency of these mixed clones will depend upon the mode of integration of the markers involved : on the basis of the proposed hypothesis for the integration of H.E. and L.E. markers, one expects 100 % of mixed clones when marker A has a low efficiency and none when it has a high efficiency, marker B having a high efficiency.

b) Strains :

The donor strains of D. pneumoniae were streptomycin and aminopterin resistant. Only one H.E. streptomycin marker was used, str r-41 (resistance to 2 mg/ml streptomycin). The aminopterin markers belong to the same locus ami A (resistance to 10^{-5} M aminopterin) : ami A-r1 is an H.E. marker, ami A-r5 and ami A-r11 are L.E. markers and located on each side of ami A-r1.

c) Isolation of clones :

The media and the basic transformation techniques have been described in an earlier publication (Sicard, 1964). Competent wild-type cells were treated with a saturating concentration of transforming DNA for 15 mn. Glycerin was then added immediately to the cells to give a final concentration of 12 % and the DNA-bacterial complexes stored at -70°C. Aliquots of such complexes were thawed and spread by filtration onto a large Millipore membrane. The membrane was laid on the surface of a non-selective medium poured in a Petri dish for complete phe-

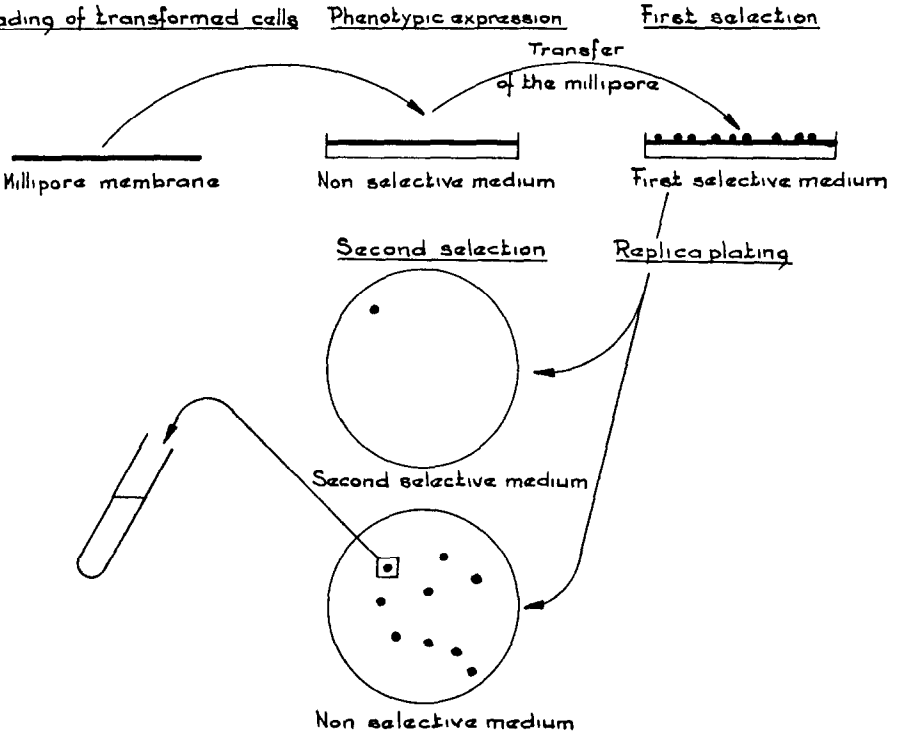

Figure 2 : Isolation of doubly transformed clones

notypic expression. After 2 1/2 hours incubation at 37°C, the filter was transferred to a plate containing the first selective medium and incubated overnight. The hundred or so colonies which appeared on the surface of each filter were replicated onto a plate containing the second selective medium and onto a control plate without selective agent. After incubation, doubly transformed clones were identified and picked from the control plate. The composition of these clones were then determined by replica-plating (figure 2).

RESULTS AND DISCUSSION

Aliquots of the same batch of competent cells were treated with several samples of DNA, one sample bearing ami A H.E. and str r-41 markers, and each of the others bearing a different ami A L.E. marker with the same str r-41 marker. The first selection was made for aminopterin resistance. The results of the clonal analysis are presented in table 1.

Transformation n°	ami A marker	Observed numbers pure	mixed	Percentage of mixed clones
1	r1 (HE)	38	8	17
"	r5 (LE)	18	21	55
"	r11 (LE)	20	26	56
2	r1 (LE)	32	7	17
"	r11 (LE)	13	32	71

Table 1 : Comparison between H.E. and L.E. ami A markers.
The average composition of a mixed clone is about 50 % of the doubly transformed bacteria.

There were many more mixed clones with a L.E. than with an H.E. marker. This is in good agreement with the hypothesis proposed by Ephrussi-Taylor (1966) : L.E. markers modify both strands of the recipient molecule while H.E. markers modify only one. However, mixed clones observed with ami A H.E. marker, as well as pure clones with ami A L.E. markers were unexpected. Several phenomena could explained such discrepancies :

1) Mixed clones could be due to independent transformation of two cocci of the same pneumococcal chain. However, this could only account for a low base-line frequency of mixed clones, less than 6 %, since, in these experiments, all the bacteria were transformable and the transformation rate for a H.E. marker was about 6 % per colony-forming unit.

2) L.E. markers might be integrated occasionally via a single-stranded mechanism. On the other hand, genetic information of H.E. markers might sometimes be transmitted into the two strands of the recipient molecule.

3) Since transformation in pneumococcus occurs during the exponential phase of growth, DNA replication could modify the composition of the progeny in the two following cases, A being the first selected marker (Figure 3) :

a) When markers A and B are L.E. and H.E. respectively, and marker A is already replicated, the progeny would be pure ;
b) when both markers are H.E. markers, but marker B is replicated before A, the progeny would be mixed.

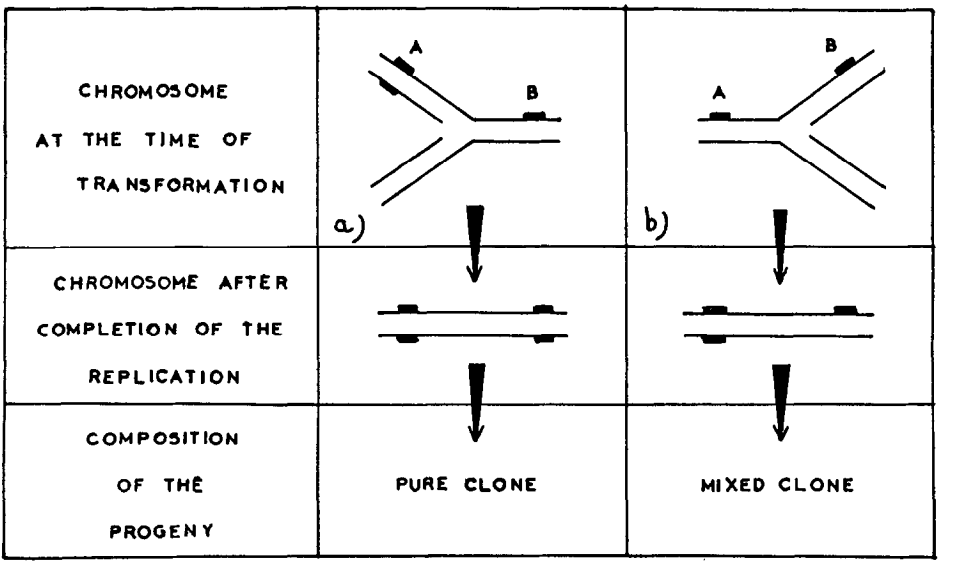

Figure 3 : Influence of the replication on the progeny of doubly transformed cells, marker A being selected first.

If there is a polarized replication in pneumococcus as well as in E. coli and B. subtilis, these two possibilities a) and b) are mutually exclusive.

Experiments have been carried out to determine if a locus is replicated before the other. For this purpose, markers of identical efficiency have been chosen. Mixed clones are expected only in the case presented in figure 3b. Thus, if the replication is polarized, only one of the two orders of selection (A first or B first) will lead to mixed clones, the reverse order leading only to pure clones. The probability of a replication point between the two markers will depend upon their distance on the chromosome and the frequency of mixed clones will therefore be related to the genetic distance if transformation and replication are two independent processes.

Wild-type cells were transformed with DNA bearing an ami A H.E. marker and str r-41 marker and the frequencies of mixed clones were estimated, as related to the order of selection (either resistance to aminopterin or to streptomycin first). The results are presented in table 2.

First selection : streptomycin			First selection : aminopterin		
pure	mixed	% mixed clones	pure	mixed	% mixed clones
200	85	30 ± 5	157	31	16 ± 6

Table 2 : Frequencies of mixed clones as related to the order of selection when using two H.E. markers.

There was a significant difference in the frequencies of mixed clones depending upon the order of selection. Our interpretation is that the ami A locus must be replicated before the str locus, the two loci belonging to the same replicon (Jacob, Brenner & Cuzin, 1963). Thus transformation and replication seem to be two independent processes. This is in agreement with the results obtained by Archer & Landman (unpublished results) in B. subtilis but in contradiction with the assumption presented by Bodmer (1965) using the same organism.

Since ami A locus is replicated before str locus, some pure clones are expected when the first selected marker has a low effi-

ciency (figure 3a and table 1). The majority of mixed clones observed in table 2 when aminopterin resistants were selected first, was probably due to a base-line level of L.E.-like integration for H.E. markers.

Acknowledgements : We wish to thank Miss A.M. Melot for her able technical assistance, Dr. W. Guild for helpful discussion and Dr. L. Butler for his help in preparing the manuscript. This work was supported by a grant from the "Délégation Générale à la Recherche Scientifique et Technique".

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