IDENTICAL INITIAL STEPS DURING TRANSFORMATION FOR HIGH AND LOW EFFICIENCY MARKERS IN Diplococcus pneumoniae

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Received June 24, 1968

In bacterial transformation, donor DNA induces genetic modifications of the chromosome of the recipient organism. In Diplococcus pneumoniae, the efficiency of the DNA in transformation depends upon the markers, the markers falling into two classes : high (H.E.) and low (L.E.) efficiency markers (Sicard, 1964 ; Ephrussi Taylor, Sicard & Kamen, 1965). The recombination processes are different for these two classes since H.E. markers modify only one of the chains of the recipient chromosome while L.E. markers transfer their information to both strands, which are therefore used as templates for the DNA replication (Ephrussi-Taylor, 1966 ; Louarn & Sicard, 1968). Recombination mechanisms might differ :

a) - at the first steps of the transformation : the two strands of donor DNA might be simultaneously integrated for L.E. markers, while it has been demonstrated that only one strand is integrated for H.E. markers (Lacks, 1962 ; Guild & Robinson, 1963 ; Fox & Allen, 1964) :

b)- at later steps : only one strand might be integrated for L.E. as well as for H.E. markers ; then, for L.E. markers, the chromosome might become rapidly homoduplex, independently of the normal replication cycle, by excision of the parent complementary segment and repair of the eliminated fragment (Ephrussi-Taylor, 1966).

This report presents the results of transformation experiments performed with heteroduplex DNA, only one strand of which bears the genetic information coming from H.E. or L.E. markers. The behaviors of these two kinds of markers were found to be identical and we conclude that only one strand of donor DNA is involved in transformation for L.E. markers.

MATERIALS AND METHODS

a) Principle :

The method consisted of a comparison of the relative efficiency of a L.E. marker to an H.E. reference marker before and after denaturation and renaturation of the donor DNA. A mixture of a DNA bearing two independent genetical markers of low and high efficiencies with a large excess of a wild-type DNA was heat-denatured and reannealled. The renaturation would occur at random (Marmur, Schildkraut & Doty, 1962) and fragments bearing the genetic markers would be mostly hybrids after this treatment.

Penetration of such an hybrid particule will lead to a genotypically transformed pneumococcus only if the strand bearing the marker is integrated. Thus, if both strands of the DNA are integrated, the number of transformed cells produced could be twice that obtained if only one strand is used at random.

MARKER USED	BEFORE UPTAKE	GENETIC MODIFICATION	
		IF THE INTEGRATION IS SINGLE STRANDED FOR H.E. L.E.	IF THE INTEGRATION IS DOUBLE STRANDED FOR L.E.
L.E.	M Hybrid DNA Recipient Cell	M OR	M
H.E.	M Hybrid DNA Recipient Cell	M OR	NOT INTEGRATED VIA THIS MECHANISM
TRANSFORMATION RATIO L.E. / H.E. EXPECTED :		0.1 : 1	0.2 : 1

Figure 1 : Expected efficiencies in transformation with hybrid DNA.

In this experiment, the relative efficiency measured by the ratio of transformed cells for the L.E. marker to the transformed cells for the H.E. marker will depend upon the mode of integration of the L.E. marker : if the two strands of donor DNA are integrated for a L.E. marker (hypothesis a), the relative efficiency will increase by a factor 2 when the donor DNA is hybrid ; if only one strand of donor DNA is integrated for a L.E. as well as for a H.E. marker (hypothesis b), the relative efficiency will remain unchanged, the donor DNA being native or hybrid (Figure 1).

b) Markers :

str-r 41 : resistance to 2 mg/ml streptomycin, the H.E. reference marker ; ami A-r 9 (L.E.) and ami A-r 1 (H.E.), two linked markers belonging to the same ami A locus (resistance to 10^{-5}M. aminopterin).

c) Preparation of hybrid DNA :

DNA was extracted from the following strains :

— wild-type strain (C1 3)
— wild-type reversion from ami A-r 9 (9 rev)
— ami A-r9 str-r 41 (9 S)
— ami A-r1 str-r 41 (1 S)

The DNA solutions were prepared by a modified method of Marmur (1961). They were deproteinized twice and kept frozen in Saline-Citate solution. To get artificial hybrids, the solutions of DNA were mixed in 0.4 M NaCl pH 7.2 to give final concentrations of 0.2 µg per ml. for the genetically labelled DNA and 8 µg. per ml. for the wild-type DNA. The mixture was heated 10 mn. at 100° and reannelled for 3 hours at 60°.

d) Transformation procedure :

Methods for the transformation of D. pneumoniae have been described previously (Sicard, 1964). Competent cells (1 ml) were treated with a solution of transforming DNA (0.1 µg) for 15 mn. The bacteria were plated in non selective medium for complete phenotypic expression. After 2 1/2 hours incubation at 37°, a second layer of nu-

trient agar containing the antibiotic was poured on the surface for selection, and the plates incubated overnight. The number of resistant colonies was then determined.

RESULTS AND DISCUSSION

The results are presented in table 1a. The relative efficiency of ami A-r 9 (L.E.) to str-r 41 (H.E.) remained constant, whether the donor DNA was native or hybrid. This is in good agreement with the idea that only one strand of donor DNA is involved for L.E. markers.

DNA mixture wild-type	mutant	Treatment	Percent str-r41 residual activity	Efficiency ami A-r / Str-r
a) C1 3	9 S	control	100	0.15
"	"	denatured	6	-
"	"	renatured	49	0.14
b) C1 3	1 S	control	100	1.24
"	"	denatured	2	-
"	"	renatured	60	1.27
c) 9 rev	9 S	control	100	0.14
"	"	denatured	3	-
"	"	renatured	48	0.15

Table 1 : Transformation efficiencies of hybrid DNA

However, this could be irrelevant if the extent of the renaturation is lower either a) in the ami A region than in the str region ; b) for a strand bearing a L.E. marker hybridizing with one bearing an H.E. allele.

To exclude the first possibility, we have performed experiments with a DNA bearing an H.E. ami A marker (ami A-r 1) closely linked to ami A-r 9. The results present no evidence that the efficiency of integration is modified by denaturation and renaturation (table 1b). The two regions ami A and str showed the same ability for renaturation.

The second possibility was eliminated by experiments involving another wild-type strain, 9 rev, which is a revertant of ami A-r 9. Whereas the reversion occured at the actual site r 9, it does not possess exactly the same properties as the original wild-type strain C1 3 : the efficiency of ami A-r 9, low when tested on C1 3, is now high when tested on 9 rev as a recipient strain, and this change occurs only for this marker (Sicard & Ephrussi-Taylor, 1966). Thus, the hybrid formed by a strand bearing the r 9 site with the complementary strand bearing the 9 rev site would no longer imply a low efficiency configuration. Table 1c shows that the replacement of C1 3 by 9 rev as the wild-type DNA did not modify the results.

These data, together with the results of clonal analysis presented in a previous paper (Louarn & Sicard, 1968) led us to the following conclusions for the integration of transforming DNA : The first steps of the transformation seem identical for H.E. and L.E. markers, as far as both cleavage of the double-stranded donor DNA into two single strands and the initial pairing of one of the strands with the recipient genome are concerned. However, whilst with H.E. markers it is only necessary to replace one of the recipientstrands with the homologous donor, the complete integration of L.E. markers requires also the excision and repair of the opposite strand, so that an homoduplex structure is obtained. Therefore, "permissive" and "non-permissive" confrontations between donor and recipient bases could control the process of recombination. How a "non-permissive" confrontation leads to a low level of integration is still unknown.

Acknowledgements: we wish to thank Miss A.M. Melot for her able technical assistance, 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."

REFERENCES

Ephrussi-Taylor, H., Sicard, A.M. & Kamen, R., (1965), Genetics, 51, 455.
Ephrussi-Taylor, H., (1966), Genetics, 54, 211.
Fox, M.S. & Allen, M.K., (1964), Proc. Nat. Acad. Sci. U.S., 52, 412.

Guild, W.F., & Robinson, M., (1963), Proc. Nat. Acad. Sci. U.S., 50, 106.
Lacks, S., (1962), J. Mol. Biol., 5, 119.
Louarn, J.M. & Sicard, A.M., (1968), Biochem. Biophys. Res.Comm., 30, 683.
Marmur, J., (1961), J. Mol. Biol., 3, 208.
Marmur, J., Schildkraut, C.L., Doty, P., (1962), Symp. Fundamental Cancer Res. 15, 9, Houston.
Sicard, A.M. & Ephrussi-Taylor, H., (1966), C. R. Acad. Sci. Paris 262, 2305.