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Number of Transformable Units Per Cell in *Diplococcus pneumoniae*

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Analysis of frequencies of single and random multiple transformations in *Diplococcus pneumoniae* showed that there are *at least two* transformable units per cell of the *total* population in highly competent cultures. If 100% of the cells are competent in these cases, the units may be interpreted as the strands of one duplex deoxyribonucleic acid recipient chromosome. The theory is developed to allow for extension to more complex situations.

When highly competent cultures of transformable bacteria are exposed to deoxyribonucleic acid (DNA), there are often fewer cells multiply transformed for unlinked markers than predicted on the simplest model of random interaction of DNA fragments with uninucleate cells. Such data lead to calculated fractions of competent cells exceeding 100% (1, 4, 8, 9). Cahn and Fox (1) suggested that this result might be caused by segregation of markers inserted into opposite strands of the recipient chromosome, rather than into a single transformable structure as is implicit in the formulation of Goodgal and Herriott (6). Singh and Pitale (13) used a two-strand model to analyze data on competent *Bacillus subtilis*, but because the level of competence was low they could not prove its validity.

We have seen the same result in *Diplococcus pneumoniae*, and in this paper we show that there are at least two recipient chromosomal units per average cell of the total population in highly competent cultures. These must be interpreted either as the DNA strands or as duplicated chromosomes (nuclei), and the evidence for single-strand information transfer heavily favors the former. The formal analysis is extended to include results expected for replicating chromosomes, where some markers may be represented by four or more strands.

MATERIALS AND METHODS

The strains and materials used have been described elsewhere (2).

Competent cultures of strain Rx-1, a drug-sensitive recipient, were routinely prepared as follows. Cells were subcultured in CAT medium (2) for one or two cycles of 100-fold exponential growth at 37 C. When the optical density reached 0.08 (Coleman Junior spectrophotometer, 18-mm tube, 600 nm), representing 5×10^8 cells/ml, they were diluted 100-fold into 280 to 500 ml of transforming medium (CAT plus 0.2% bovine albumin and 10^{-3} M $CaCl_2$), and were incubated for 75 to 105 min, depending on recent experience. At this time 10% glycerol was added, and the culture was divided into 10- to 25-ml portions in screw-cap tubes, frozen in dry ice-acetone, and stored at −22 C. For transformation, a tube was thawed in a bath at 37 C for 12 min and mixed gently by inversion; samples were then added to 0.1 volume of DNA solution.

In the experiments reported here, two different preparations were exposed to excess multiply marked DNA molecules from strains 5MC (experiment 1) and 8M (experiment 2), at 30 C. At 15 min, deoxyribonuclease was added; the cells were diluted, plated in an agar overlay on blood-agar plates, incubated at 37 C for 2 hr for expression, overlaid with agar containing the various drugs, and incubated for 36 hr before the colonies were counted. Final drug concentrations for these experiments were as follows: streptomycin, 50 μg/ml; erythromycin, 0.2 μg/ml; bryamycin, 0.3 μg/ml; and novobiocin, 2 μg/ml. Similar results were seen at substantially higher drug concentrations, and this fact and the consistency of the results among various marker combinations argue that we were not losing multiple transformants in the assay procedure.

Theory. If a single chromosome per cell were modified in both strands of the DNA, or if only one strand were transformable, the expected number of random double transformants per milliliter would be

$$n_{1,2} = \frac{n_1 n_2}{f_c n_0} \qquad (1)$$

where the subscripts refer to markers, and f_c is the fraction of total cells, n_0, which are competent (6). Similarly, for random triples,

$$n_{1,2,3} = \frac{n_1 n_2 n_3}{f_c^2 n_0^2} \qquad (2)$$

One needs a factor q multiplying f_c, however, to allow for a multiplicity of transformable units per cell, either chromosomes (duplex DNA) or single DNA strands. If the donor strands are equally efficient and modify only one strand of the recipient, the simplest situation is to expect $q = 2$, leading to one-half as many doubles and one-fourth as many triples as expected on the basis of equations 1 and 2. Without this factor, one overestimates the number of competent cells.

Because it is only the product $f_c q$ which may be calculated from the data, the least prejudicial forms of the relations are, for doubles,

$$f_c q_d = \frac{n_1 n_2}{n_{1,2} n_0} \tag{3}$$

and for triples,

$$f_c q_t = \sqrt{\frac{n_1 n_2 n_3}{n_{1,2,3} n_0^2}} \tag{4}$$

The units of $f_c q$ are roughly interpretable as transformable units per cell of total population. The subscripts d and t imply that the values of q may differ, as will be seen later. When f_c is low, the necessity for a factor q may not be detectable. It is essential, of course, that n_0 represent total cells rather than colony-forming units.

RESULTS AND DISCUSSION

Table 1 lists results for single and random multiple transformations in *D. pneumoniae* for four drug-resistance markers, along with total cell titers, determined by counting in Petroff-Hausser chambers, and the $f_c q$ factors calculated from equations 3 and 4. (The *str-r* and *ery-r* markers are distantly linked, but not sufficiently to affect these data.) Similar results were obtained in other experiments not listed.

Because f_c is a constant in a given culture and cannot exceed one, it is clear that there are at least two recipient structures per cell. Although in principle these could be separate chromosomes, the fact that single-strand displacement is well established in transformation (5, 7, 10) and that the strands are of equal efficiency (3, 12) leads us to interpret the results as representing approximately 100% competent cells with an average of about one recipient duplex DNA per marker. If there were two nuclei per cell, however, f_c could, e.g., be 0.5 and $q = 4$. Singh and Pitale (13) found uninucleate *B. subtilis* to predominate in the competent fraction. An implication of the fit to the model is that the recipient sites are equally likely to be transformed in any competent cell.

On further analysis of the situation, however, it should be noted that in a replicating chromosome, of defined origin, one should expect the value of q to be greater than 2 in some cases, depending on the loci of the markers scored, as discussed in Fig. 1.

Note that even if one of a pair of markers is near the origin, and therefore usually represented by four strands, q will be 2 if the second marker

TABLE 1. *Apparent transformable units per cell estimated from numbers of random multiple transformants in D. pneumoniae*

Determination	Expt 1		Expt 2	
	Cells/ml	$f_c q$[a]	Cells/ml	$f_c q$[a]
Total cells[b]	3.77×10^7		3.75×10^7	
Transformants[c]				
Singles:				
str-r	2.42×10^6		1.84×10^6	
ery-r	2.31×10^6		1.67×10^6	
nov-r	2.06×10^6		1.50×10^6	
bry-r	—		1.54×10^6	
Doubles:				
str-ery	7.10×10^4	2.09	4.05×10^4	2.03
str-nov	7.05×10^4	1.88	3.75×10^4	1.96
ery-nov	6.20×10^4	2.04	4.00×10^4	1.67
bry-str	—	—	4.45×10^4	1.70
bry-nov	—	—	4.15×10^4	1.50
Triples:				
str-ery-nov	1.75×10^3	2.1	7.3×10^2	2.1
str-nov-bry	—	—	6.8×10^2	2.1
ery-nov-bry	—	—	6.7×10^2	2.0

[a] Apparent transformable units per cell, from equations 3 and 4.
[b] Mean of five microscopic counts in a Petroff-Hausser chamber.
[c] DNA concentration was 5 μg/ml in expt 1 and 1 μg/ml in expt 2.

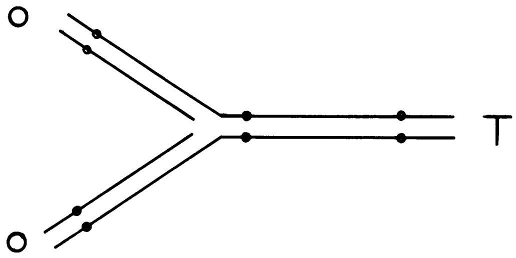

FIG. 1. *Expected probabilities of random multiple transformation as a function of marker position, for single-strand conversion. Doubles: (i) for one marker near terminus, $q_d = 2$, independent of locus of 2d marker; (ii) for one marker near origin, q_d rises from 2 to 4 as locus of 2d marker varies from terminus (T) to origin (O). This whole analysis breaks down when markers are close enough to be co-transferred on one DNA fragment, i.e., when multiple transformation is no longer random. Triples: (iii) for two markers near T, $q_t^2 = 4$ for any locus of 3d marker (equivalent to q_d^2 in (i) above); (iv) for two markers near O, q_t^2 rises from 8 to 16 as the 3d marker locus varies from T to O [$q_t^2 = 4q_d$ where q_d varies from 2 to 4 as in (ii)]; (v) for one near T and one near O, q_t^2 varies from 4 to 8 as third marker locus varies from T to O [product of q_d's in (i) and (ii)]. For any marker for which there exists a probability p of modifying both strands of the recipient (11), q decreases by a factor 2p. The q's are expected to depend on the marker combination used to evaluate them. A more general notation is $q_d \equiv q_{ij}$, and $q_t^2 \equiv q^2_{ijk} \equiv q_{ij} \cdot q_{ij,k} = q_{ik} \cdot q_{ik,j} = q_{jk} \cdot q_{jk,i}$.*

is near the terminus, because the replication fork will pass the latter before segregation occurs. For a chromosome with one replicating fork, the maximum value of q would be 4 if all markers scored were near the origin. In principle, one might be able to construct a rough map from such data. The fact that in Table 1 no q exceeds 2 by a large factor suggests that at least three of the markers are closer to the terminus than to the origin. Without independent evidence that $f_c = 1.00$, however, this conclusion is not rigorous.

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