Transformation in Group H Streptococci: Blackburn Strain Transformable Mutants

BOHDAN STAROŚCIAK, JANUSZ NAWŁĘCZ,¹ AND WŁADYSŁAW T. DOBRZAŃSKI

Department of Molecular Genetics, University Medical School, Warsaw, Poland

Received for publication 27 December 1971

Transformable mutants of the nontransformable Blackburn strain group H streptococci have been isolated. In contrast with the wild strain, the cell surface extracts of these mutants contain the factor inactivating deoxyribonucleic acid *in vitro*.

The serological group H of streptococci includes, among others, the strains Challis, Wicky, Blackburn, and Channon.

As we have recently reported (1, 4, 5), the appearance of competence in Challis and Wicky strains depends both on the presence of the competence factor (cpf) and on a certain nuclease (deoxyribonuclease). This nuclease (iF) seems to be localized in the surface cell layers which, however, are not accessible to proteolytic enzymes. The presence of iF was demonstrated in alkaline cell surface extracts by measuring *in vitro* the inactivation of transforming deoxyribonucleic acid (DNA) by these extracts. It was also reported that Blackburn and Channon strains, hitherto regarded as nontransformable, produce considerable amounts of cpf; iF activity could not be found in these strains (1, 3).

In the experiments reported herein, transformable mutants of Blackburn strain were sought; an attempt was also made to determine whether they differ in iF production from the wild strain.

Wild Blackburn (cpf⁺, iF⁻, antibiotic-sensitive) in log phase was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (20 to 40 µg/ml) for 30 min at 37 C. The cells were then washed and incubated in broth containing 3% horse blood overnight. Samples from these cultures were subjected, as previously described (3), to transformation with Challis DNA containing *str-r* marker. Thereafter, the samples were inoculated on blood-agar plates with 250 µg of streptomycin per ml; 150 streptomycin-resistant colonies were isolated from the plates. All clones were tested qualitatively for transformability by the second-step transformation, using the same method and DNA from

a spontaneous rifampin-resistant (*rfp-r*; 3 µg/ml) Challis strain mutant. After 120 min of phenotypic expression, the transformants were isolated on blood-agar plates containing 3 µg of rifampin per ml.

Among 150 clones, 48 were transformable. Four of these were tested for the presence of cpf and iF in cell surface extracts and for transformability by using homologous Blackburn DNA from a spontaneous *rfp-r* mutant of this strain. Isolation of cpf and the determination of its activity were described previously (2, 5).

Data in Table 1 illustrate growth of the population and the appearance of competence measured by the transformation yield in the transformable *str-r* mutants of Blackburn strain, as compared with the wild Challis strain. The presence of cpf and iF in the mutants is presented in Table 2.

Data from the control samples showed the following: (i) samples in which deoxyribonuclease was added to the culture at the same time as DNA showed no transformation; and (ii) iF could not be found in the wild Blackburn strain and its 12 clones.

Data presented in the tables show that: (i) transformable mutants occur in Blackburn strain, and some of them exhibit high transformability; (ii) the competence peak occurs later in Blackburn mutants than in the wild Challis strain; and (iii) in contrast to the wild, nontransformable Blackburn strain, its transformable mutants showed the presence of iF in cell surface extracts. This finding supports our observations on the role of this nuclease in competence and transformability of group H streptococci (1, 3, 4).

When transformable Blackburn mutants are transformed in the same experiment, with *rfp-r* Challis and Blackburn DNA, the yield of re-

¹ Deceased.

TABLE 1. Growth and competence measured by transformation yield of *rfp-r* marker in transformable mutants of Blackburn strain and in Challis strain^a

Strain	Incubation time (min) of cells before DNA addition									
	90		120		150		180		210	
	G ^b	C ^c	G	C	G	C	G	C	G	C
Blackburn mutant 51	14	0.02	50	0.1	80	6.2	220	0.6	880	0.04
Blackburn mutant 70	71	0.015	176	0.01	320	1.4	872	0.005	988	0.0002
Blackburn mutant 124	40	0.007	116	0.005	232	0.2	576	0.4	1,080	0.02
Blackburn mutant 135	48	0.008	116	0.065	176	0.9	560	0.06	1,072	0.001
Wild Challis	55	0.1	212	2.2	236	1.6	480	0.85	1,200	0.1

^a Method of transformation (3): about 5×10^6 cells/ml were incubated in HE medium before addition of Blackburn DNA with *rfp-r* marker (final concentration, 2 μ g/ml) for various time periods; after 15 min of contact of DNA with cells, the reaction was stopped by deoxyribonuclease ("Streptodornase," Biomed, Poland; 25 units/ml) treatment, and the cells were incubated for an additional 95 min before plating on selective medium containing rifampin (3 μ g/ml).

^b G, growth; colony-forming units per ml $\times 10^6$.

^c C, competence; measured by transformation yield (%).

TABLE 2. Presence and activity of competence factor (*cpf*) and nuclease (*iF*) in transformable mutants of the Blackburn strain

Strain	Cpf ^a	iF ^b
Blackburn mutant 51	+	70
Blackburn mutant 70	+	83
Blackburn mutant 124	+	73
Blackburn mutant 135	+	68
Blackburn wild strain (control 1)	+	0
Challis (control 2)	+	84

^a Cpf activity in cell extracts (biological units), as determined by competence induction in Wicky strain (5).

^b iF activity in cell extracts, expressed as per cent inactivation in vitro of transforming DNA after 90 min of contact, as compared with activity of untreated DNA (3).

action is markedly higher in the sample treated with homologous DNA.

Transformation of the Blackburn strain seems to provide a suitable system for studies on competence and on the role of iF and cpf in competence.

Among group H streptococci, strains cpf⁺,

iF⁺; cpf⁻, iF⁺, and cpf⁺, iF⁻ are now available, and cpf⁻, iF⁻ cells can be cultivated.

We are very grateful to D. Shugar and M. Piechowska for reading the manuscript and for the technical assistance of M. Lipska and M. Emanuel.

This investigation was supported by a grant from the Polish Academy of Sciences (09.3.1./1.2.5.).

LITERATURE CITED

1. Dobrzański, W. T. 1971. Transformation among streptococci: mechanisms and implications. In *Streptococci and streptococcal diseases—Symposium*, Minneapolis, May, 1971. Academic Press Inc., New York, in press.
2. Dobrzański, W. T., and H. Osowiecki. 1967. Isolation and some properties of the competence factor from group H Streptococcus strain Challis. *J. Gen. Microbiol.* 48:299-304.
3. Nałęcz, J., and W. T. Dobrzański. 1972. Correlation between the occurrence of competence in the transformation of group H streptococci and the presence of the competence factor and *in vitro* DNA-inactivating factor. *Mol. Gen. Genet.* 114: 249-260.
4. Nałęcz, H., H. Osowiecki, and W. T. Dobrzański. 1970. A factor inactivating *in vitro* transforming DNA isolated from transformable group H streptococci and its relation to competence of the cells. *Bull. Acad. Pol. Sci. Cl. II.* 18:687-691.
5. Osowiecki, H., J. Nałęcz, and W. T. Dobrzański, 1969. The mechanism of competence in the transformation of streptococci of serological group H. Purification and some properties of the competence factor. *Mol. Gen. Genet.* 105:16-20.