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Evidence that TraT interacts with OmpA of *Escherichia coli*

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The OmpA protein is one of the major outer membrane proteins of *Escherichia coli*. Among other functions the protein serves as a receptor for several phages and increases the efficiency of F-mediated conjugation when present in recipient cells. TraT is an F-factor-coded outer membrane lipoprotein involved in surface exclusion, the mechanism by which *E. coli* strains carrying F-factors become poor recipients in conjugation. To determine a possible interaction of TraT with OmpA, the influence of TraT on phage binding to cells was measured. Because TraT inhibits inactivation of OmpA-specific phages it is suggested that TraT interacts directly with OmpA. Sequence homology of TraT with proteins 38, the phage proteins recognizing outer membrane proteins, supports this finding. A model of protein interactions is discussed.

TraT protein *OmpA protein* *Protein interaction*

1. INTRODUCTION

Bacterial conjugation is defined as the unidirectional transfer of DNA from an F^+ donor cell to F^- recipient cells. Surface exclusion is the mechanism whereby cells carrying a sex factor are reduced in their ability to act as recipients of DNA from other donor cells carrying the same or related sex factors [1]. Among the F-like sex factors, four surface exclusion systems can be distinguished [2,3]. The mechanism of surface exclusion is not clear.

Genetic analyses of the phenomenon has shown that two genes, *traS* and *traT*, are involved in surface exclusion [4]. Mutation of either cistron can only partially abolish surface exclusion, because each cistron results in a reduction of recipient ability independent of the other. TraS, the gene product of *traS*, is a cytoplasmic membrane protein and prevents DNA transfer into the $traS^+$ cells [5]. The *traT* gene product, TraT, has been identified as an outer membrane protein and reduces the ability of $traT^+$ cells to form stable mating aggregates [5,6]. In addition, TraT is known to mediate bacterial resistance against killing by mammalian complement [7]. A major outer membrane protein, OmpA, which serves as a receptor for different T-even-like phages (like K3 and Ox2) is required in the recipient cell for efficient conjugation [8]. It has been suggested that OmpA acts as a receptor during mating aggregation [9]. A phenotypic map of OmpA mutants [10] showed that the regions responsible for F-conjugation and phage receptor function are overlapping.

So far, no direct evidence exists that TraT abolishes the formation of stable mating aggregates by interaction with the OmpA protein. Here, this evidence is presented. Whenever TraT is present in the outer membrane of *E. coli*, inactivation of OmpA-specific phages is effected whereas inactivation of other phages is not. Thus, there is an interplay between TraT and the tip of the long tail fibers of the T-even-like phages in OmpA binding.

2. MATERIALS AND METHODS

2.1. *Bacteria, plasmids and phages*

The bacteria and plasmids are listed in table 1. The phages used are all of T-even type. T2 uses the

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outer membrane protein OmpF as receptor [13]. K3 and Ox2 use OmpA as receptor [8]. K3hx is a host range mutant of K3, which uses OmpA as receptor but a different part of it [14].

2.2. *Inactivation of phages*

Bacteria were grown to 1×10^9 cells/ml in LB with the appropriate antibiotic in the case of plasmid containing strains. The cells were inactivated with $CHCl_3$ or 100 μg/ml chloramphenicol. Phages were added to the bacteria at an appropriate concentration and the mixture incubated at 35°C. At different times samples were taken and chilled in ice-cold phage buffer (10 mM Tris-HCl, pH 8.0, 10 mM NaCl, 10 mM $MgCl_2$). Remaining phages were counted on LB plates containing P400 [9].

2.3. *Computer analyses of the sequences*

To compare the sequences the program RELATE was chosen [15]. This program establishes homologies between distantly related genes and gene products. One of the outputs of the program is a number called segment comparison score (SD units). This number indicates whether homologies found are statistically significant or not. The probability of obtaining by chance SD units of 10, 3 and 1 is 0.762×10^{-23}, 0.133×10^{-2} and 0.159, respectively. SD units below 1 are thought to be not significant and numbers above 1 indicate significant homologies within the sequences. Another output of the program is the localization of the top scores. These are the sequences with the highest homology.

3. RESULTS

3.1. *Inactivation of OmpA-specific phages is inhibited by TraT*

Phages K3, K3hx and Ox2 use OmpA as a receptor. Measuring inactivation of these phages by bacteria (JC3272, table 1) containing vector DNA (pSC101) results in the inactivation rate shown in fig.1 only for phage K3hx. pRS31 is a chimeric pSC101. The insert originates from the F-transfer region and encloses genes *tra*S, T, D, I, and Z [16,17]. JC3272 (pRS31) expresses surface exclusion proteins more strongly than the F-factor itself [12] but does not influence the expression of OmpA [6].

Table 1

Bacteria and plasmids

JC3272	*traS*$^-$, *traT*$^-$	[11]
JC3272 (pSC101)	*traS*$^-$, *traT*$^-$	[12]
JC3272 (pRS31)	*traS*$^+$, *traT*$^+$	[12]
JC3272 (pBE5)	*traS*212, *traT*$^+$	[12]
JC3272 (pBE21)	*traS*$^+$, *traT*246	[12]

Only genetic markers important for this communication are indicated

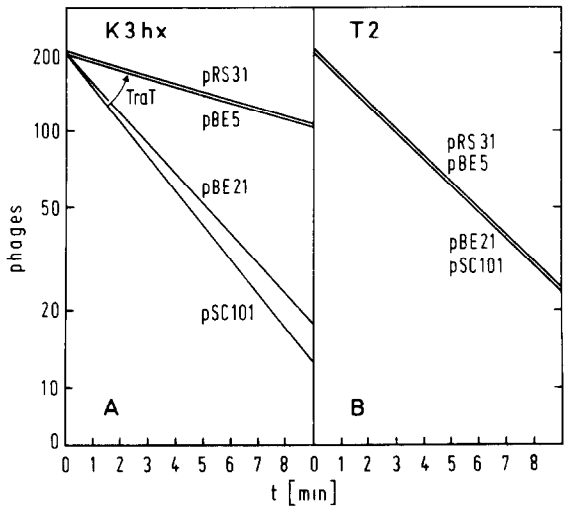

Fig.1. Inactivation of phages. JC3272 containing the plasmids indicated was tested for its ability to inactivate phages K3hx (A) and T2 (B).

The inactivation of the OmpA-specific phages by JC3272 (pRS31) is slower than inactivation by JC3272 (pSC101) (fig.1). To determine if this effect is due to the surface exclusion cistrons, mutant plasmids of pRS31, pBE5 and pBE21, were used. pBE5 contains a *tra*S mutation but still expresses TraT protein and pBE21 carries a *tra*T mutation but is still *tra*S wild type (table 1). Regarding the phage inactivation, JC3272 (pBE5) behaves similarly to JC3272 (pRS31) and JC3272 (pBE21) similarly to JC3272 (pSC101) (fig.1). Therefore the reduction of OmpA-specific phage inactivation is due to TraT.

3.2. *Inactivation of T2 is independent of TraT*

Phage T2 uses the outer membrane protein

OmpF as receptor. The inactivation of the phage by JC3272 containing either pSC101, pRS31, pBE5 or pBE21 is all the same (fig.1). Thus, expression of TraT does not affect T2 binding to the outer membrane and therefore, there is no general effect on the structure of the bacterial surface.

3.3. *Sequence of TraT is homologous to gp38 of the phages*

All phages used here recognize their cellular receptors with the tip of their long tail fibers. As adhesin, gp38 has been identified at the tip of the long tail fibers (in preparation).

The sequences of several gp38 can be deduced from the DNA sequences (in preparation). These data suggest that the receptor-recognizing domains consist of repetitive units, homologous to membrane proteins which are flanked by glycine and alanine residues. The sequences of three surface exclusion groups of TraT proteins are known. TraT of Inc F_0 *lac* [18] differs in several amino acids from TraT of R100 [19] and F. TraT of R100 and F only differ in three amino acids [18]. The DNA and protein sequences of the adhesins of phages T2 and K3 were compared to those of the TraT protein. The data illustrating the homology of the genes and proteins are listed in table 2. All values obtained (SD units) are above 1, independent of the parameters chosen. This means that the DNA and protein sequences of *traT* are

Table 2

Homologies between *traT* and T2 and K3 genes 38 and the predicted proteins

DNA	*traT*	Protein	TraT
T2 g38	5,0 (75)	T2 gp38	2,6 (MD)
	3,8 (30)		2,2 (UP)
K3 g38	6,2 (75)	K3 gp38	1,5 (MD)
	5,6 (30)		2,6 (UP)

The values indicate segment comparison scores (SD units) obtained by the program RELATE. DNA comparison: segments 75 or 30 nucleotides long (numbers in parentheses) were compared (100 random runs). The scoring matrix was the unitary matrix. Protein comparisons: segments 10 amino acid residues long were compared by using the mutation data matrix (MD) or unitary matrix (UP)

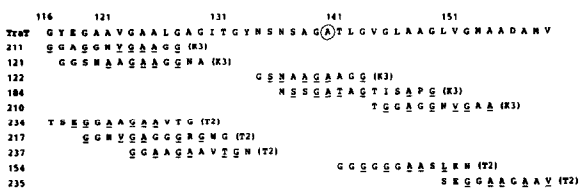

Fig.2. Sequence homologies between TraT and T2 and K3 proteins 38 found by the program RELATE. For each comparison (unitary matrix for proteins, segments 10 amino acids long, 100 random runs) five top scores were indicated. The sequence of TraT of F and R100 differs in the one amino acid encircled.

significantly homologous to g38 of phages. To determine the homologous sequences, the top scores of the proteins were aligned (fig.2). TraT amino acid residues 110–150 are homologous to T2 and K3 regions of the C-terminal part of proteins 38. This C-terminal part of g38 is the host recognizing area. In this region, TraT of the F-factor differs from TraT of R100 by only one amino acid: Ala^{140} is replaced by Gly [18]. This one amino acid exchange could determine the different specificities of these TraT proteins. R100 can transfer DNA to *ompA* mutants whereas F cannot [2]. It could be argued that an exchange of an alanine to a glycine is unlikely to be relevant for receptor recognition. A host range mutant of phage Ox2 using a different outer membrane protein of *E. coli* as receptors [20] has been sequenced. The mutation causes an insertion of a glycine in gp38 (in preparation).

4. DISCUSSION

Among other functions, the OmpA protein serves as a receptor for several phages and is required in recipient cells for efficient F-conjugation. TraT is an outer membrane lipoprotein [21] coded by the F-transfer region and responsible for surface exclusion. In this paper it is clearly shown that TraT inhibits OmpA-specific phage binding, whereas binding of T2, which uses OmpF as a receptor, is not affected. Since TraT does not influence the amount of OmpA in the outer membrane [6], it most likely interacts directly with the OmpA protein.

The sequences of the adhesins of phages T2 and K3 are known. The receptor-recognizing areas of

the proteins consist of repetitive motifs. The amino acids constituting these motifs are flanked by glycines and alanines. Point mutations that cause host range changes and recombination analysis revealed that at least a combination of three domains of the phage proteins are involved in receptor recognition (in preparation).

The same principle of protein architecture for receptor recognition has been found in a completely different phage, f1, whose adhesin, protein A, coded by gene III, is homologous to gp38 of T2 and K3. Moreover the moieties in the regions responsible for the receptor recognition are homologous with amino acids 110–150 of TraT. This suggests that this region of TraT can interact directly with the surface exposed parts of an outer membrane protein.

The OmpA protein as a phage receptor has been analyzed. From mutational alterations causing resistance to OmpA-specific phages it has been concluded that regions corresponding to amino acid residues 25, 70, 110 and 150 are surface-exposed [22]. One to three of these domains were required for phage infection. Conjugation-deficient *ompA* mutants that contain normal amounts of the OmpA protein were localized only in one region [10,23]. This suggests that the OmpA region required for F-conjugation is smaller than the region recognized by phages and that, to mediate surface exclusion, the TraT protein would presumably only have to block a small region of OmpA. This also implies that the protein region of TraT interacting with OmpA is smaller than the region of protein 38 involved in receptor recognition. In fact, the glycine-rich repetitive unit of TraT is less than 50 amino acids long, whereas the receptor-recognizing area of proteins 38 is 150 amino acids long (in preparation).

Assuming that TraT only binds to one of the parts of OmpA required for phage adsorption, this could also explain why phage binding can only be partially inhibited by TraT protein but not completely blocked.

To summarize these findings a model for the interaction of OmpA, TraT and gp38 is proposed (fig.3). The glycine- and alanine-rich region of the F-TraT protein interacts with part of the surface-exposed OmpA domains and inhibits binding of gp38 from the long tail fibers of OmpA-specific phages.

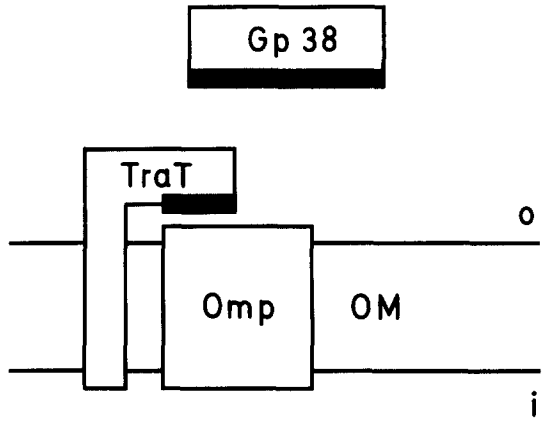

Fig.3. Model for the interplay between the lipoprotein TraT, the phage protein gp38 and an outer membrane (OM) protein.

This model may apply to TraT proteins of other surface exclusion groups. Each transferable plasmid could use a specific outer membrane protein, which, in turn, could be blocked by the specific surface exclusion protein TraT.

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