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The IncP Plasmid-Encoded Cell Envelope-Associated DNA Transfer Complex Increases Cell Permeability

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IncP-type plasmids are broad-host-range conjugative plasmids. DNA translocation requires DNA transfer-replication functions and additional factors required for mating pair formation (Mpf). The Mpf system is located in the cell membranes and is responsible for DNA transport from the donor to the recipient. The Mpf complex acts as a receptor for IncP-specific phages such as PRD1. In this investigation, we quantify the Mpf complexes on the cell surface by a phage receptor saturation technique. Electrochemical measurements are used to show that the Mpf complex increases cell envelope permeability to lipophilic compounds and ATP. In addition it reduces the ability of the cells to accumulate K^+. However, the Mpf complex does not dissipate the membrane voltage. The Mpf complex is rapidly disassembled when intracellular ATP concentration is decreased, as measured by a PRD1 adsorption assay.

Plasmids of incompatibility group P (IncP) are large self-transmissible broad-host-range plasmids carrying multiple antibiotic resistance determinants. The prototype IncP plasmid, RP4 (60,099 bp [40]), can replicate in diverse gram-negative organisms. However, the host range identified by conjugative transfer ability is considerably wider than the range of vegetative replication. Suitable shuttle vector constructions carrying the IncP transfer system allow transfer to occur from gram-negative organisms to gram-positive bacteria and to yeast (for recent review see reference 36). Evolutionary and functional relationships of the IncP DNA transfer system to other systems include Ti/Ri plasmids that direct T-DNA transfer from agrobacteria to plant cells (45). These properties make RP4 a suitable model for studying bacterial conjugation (16, 17, 24, 25, 38).

Functions responsible for the conjugative transfer of RP4 are encoded in two distinct regions on the plasmid known as Tra1 and Tra2 (Fig. 1). Essential transfer functions and the Tra1 and Tra2 core regions were recently identified and evaluated by genetic analyses (5, 29, 51). Transfer functions are divided into two groups: the DNA transfer and replication (Dtr) system and the mating pair formation (Mpf) apparatus, involved in bringing the donor and the recipient cells into intimate contact during conjugation. Dtr genes map exclusively in Tra1. This region consists of three operons and includes the origin of transfer (*oriT*), which is an intergenic region between the leader and the relaxase operons. The Dtr functions participate in relaxosome formation, the initiation complex of the transfer-replication process (37, 39, 50). Transfer of the DNA is thought to occur via TraI-piloted single-stranded intermediates that may be coated by the TraC protein (42, 52). The DNA-TraC complex is thought to be transported through a channel or pore at the mating bridge between the donor and the recipient cells. Formation of this channel or pore depends on gene products of the Mpf system. Mpf genes (*trbB*, *-C*, *-D*, *-E*, *-F*, *-G*, *-H*, *-I*, *-J*, *-K*, and *-L*) are located in Tra2 except for *traF*, which is located in Tra1.

Requirements of the Mpf system were defined by two phenotypic observations: donor-specific phage propagation, i.e., IncP plasmid-dependent phage growth, and mobilization of nonconjugative transmissible IncQ plasmid RSF1010. RSF1010 encodes its own relaxosome (2, 46). However, it relies on the Mpf transfer components of other conjugative plasmids. RSF1010 mobilization by RP4 transfer functions requires *traG* of Tra1 in addition to Mpf genes (26, 28, 29). The Mpf system (12 plasmid-encoded components) is believed to function in several processes: (i) pilus assembly and erection, (ii) contact formation and maintenance during conjugation between the donor and the recipient, (iii) DNA export into the recipient, and (iv) IncP-specific phage DNA import by injection. The proposed phage receptor complex and part of the DNA transport machinery consist of the same basic Mpf components (18, 23). Most of the Mpf components are likely to be integral membrane or membrane-associated proteins, as indicated by their amino acid sequences (27).

In this study we use the lipid-containing double-stranded DNA bacteriophage PRD1, an IncP plasmid-specific virus, to analyze the phage receptor (34). The plasmid-specified functions needed to propagate phage PRD1 only act in adsorption and DNA penetration (30). The attachment of radioactively labelled phage on the surface of the host cell provides a measure for the quantification of phage receptor complexes. The adsorption rate constant was determined to be about 3.5×10^{-10} ml/min (23), and the binding of the viruses is irreversible. The number of phage receptors on a cell is most probably a measure of the number of complexes involved in pilus extrusion and DNA transmission since the transfer genes needed for these complexes are virtually identical to those for the receptor (18, 29). The presence of a reasonably high number of phage receptors indicated that the DNA transmission structures are also abundant (23). This encouraged us to use electrochemical methods to study the permeability properties of these complexes.

Membrane voltage (transmembrane difference of electrical potential, $\Delta\Psi$) is the major component of the proton motive

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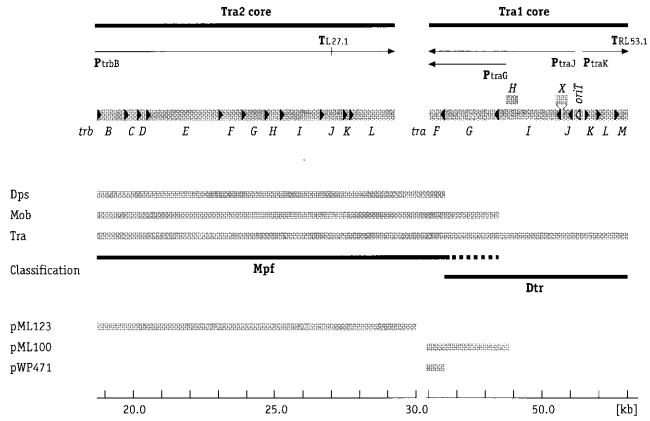

FIG. 1. Genetic organization of the Tra regions.

force (Δp), the other component being the pH gradient, ΔpH (32). ΔΨ is essential in central cellular functions such as ATP synthesis and active transport of ions and molecules across the plasma membrane (PM) (47). Membrane voltage is also a prerequisite for horizontal gene transfer in bacteria by transformation, conjugation, and transduction (for reviews, see references 10 and 35). Very little is known, however, about how ΔΨ facilitates the transport of anionic DNA molecules across the hydrophobic membrane moiety of the cell envelope. Transmission of DNA through the membrane seems to require a proteinaceous channel. However, as cells are able to carry out energy-dependent processes during conjugation as well as after DNA transfer, such a channel has hardly any severe effect on cellular energetics.

Lipophilic cations like tetraphenylphosphonium (TPP^+) are used to estimate the ΔΨ in a number of biological systems, including bacteria (22, 43). However, several other factors affect the distribution of TPP^+ between the cells and surrounding medium, making this method only qualitative (for details see Results). In spite of this, the amount of TPP^+ accumulated by the cells is still a useful parameter because of its high degree of sensitivity to changes in the permeability of the PM and in the activity of energy-transducing systems. For small objects, such as bacteria, the accumulation of TPP^+ by the cells can be measured by selective electrodes which monitor changes of the indicator ion concentration in the incubation medium (14, 21).

The bacterial PM is rather impermeable to inorganic ions such as K^+ but permeable to valinomycin-K^+ and nigericin (NG)-K^+ complexes or TPP^+. On the other hand the outer membrane (OM) porins freely exchange inorganic ions but form a permeability barrier for lipophilic compounds, such as TPP^+ and ionophoric antibiotics (for reviews, see references 19, 33, and 48). Therefore, lipophilic compounds can also be used to determine the permeability of the OMs of gram-negative bacteria. The simultaneous measurements of TPP^+ and K^+ fluxes through the bacterial envelope and the effects of ionophoric antibiotics on gradients of these ions give us valuable information about the permeability and function of both the PM and the OM.

The presence of a broad-host-range IncP plasmid-encoded DNA transfer complex and its subassemblies in the envelopes of *Escherichia coli* cells allows us to investigate the electrochemical properties of this proteinaceous DNA-translocating complex. Here we demonstrate that (i) the RP4 Mpf system forms the PRD1 receptor and is sufficient for phage DNA injection, (ii) the presence of the Mpf component in the cell envelope greatly increases cell permeability to lipophilic compounds, K^+ and ATP, and (iii) the cells containing the entire DNA transport complex are less permeable than those containing the Mpf components only.

MATERIALS AND METHODS

Bacteria, phage, plasmids, and growth conditions. The bacteria, phage, and plasmids used in this study are listed in Table 1. The strains were grown in Luria-Bertani broth (44) at 37°C with aeration. When appropriate, ampicillin sodium salt (150 μg/ml), kanamycin sulfate (25 μg/ml), or chloramphenicol (10 μg/ml) was added to the growth medium for the overnight cultures. The final cell batch was grown from diluted overnight cultures (2×10^8 cells/ml) in the absence of antibiotics. Cells were harvested at 10^9 cells/ml.

Quantification of PRD1 receptor complexes on the cell surface. Bacteriophage PRD1 was grown, labelled with a ^{14}C-amino acid mixture (6 μCi/ml; CFB.104; Amersham), concentrated, and purified by rate zonal sucrose gradient centrifugation as previously described (23). The sucrose gradient zone containing the infective viruses was stored in aliquots at −80°C in the presence of 20% glycerol. The virus titer was approximately 2×10^{11}/ml, and the specific radioactivity was approximately 2.1×10^{-5} CPM/PFU. One hundred-microliter aliquots of cells (10^9 cells/ml) were mixed with the virus preparation to obtain adsorption mixtures with multiplicities of infection (MOI) of about 20, 100, 200, and 300 at 37°C. Care was taken to keep the cell density constant in each experiment. After an adsorption time of 15 min the cell and supernatant fractions were separated by centrifugation and the cells were washed twice with 0.5 ml of Luria-Bertani broth. The radioactivity levels in the supernatant fractions and in the cell pellet were determined by liquid scintillation counting. The number of cell-associated virus particles was calculated as previously described (23).

Measurements of ion fluxes and determination of membrane voltage and ATP content. For the ion flux experiments the cells were collected at a density of approximately 10^9 cells/ml, concentrated 100-fold by centrifugation, and resuspended into 50 mM Tris, pH 8.0. The cells were kept on ice until used (maximally 4 h). The measurements of ion fluxes and the ATP content were carried out as described previously (7). The internal ion concentrations were calculated from the external one, assuming that 100 Klett units of optical density (A_{540}) corresponds to 5×10^8 *E. coli* cells/ml, that 2×10^9 *E. coli* cells correspond to 1 mg of dry mass, and that the intracellular water volume of *E. coli* is 1.1 ml/g of dry mass (3). The ΔΨ values were calculated from a modified Nernst equation, as described previously (7).

TABLE 1. Bacterial strains, phage, and plasmids

Strain, phage, or plasmid	Genotype and/or phenotype	Reference
Bacteria		
E. coli K-12		
AN180	F$^-$ *argE3 thi-1 strA*	20
KO1489	An SDS-sensitive[a] derivative of MC4100	15
JE2571	*leu thr fla pil* Sm^r	2
S. typhimurium LT2 DS88	SL5676 Sm^r (pML2)	1
Phage PRD1	wt[b]	34
Plasmids (copy number)		
RP4 (6)	Ap^r Km^r Tc^r	6
pLM2 (6)[c]	Ap^r (am) Km^r Tc^r (am)	31
pML123 (55)[d]	Tra2 *trbB-trbM* Cm^r	29
pWP471 (50)[e]	*traF* Ap^r	51
pML100 (50)[f]	*traG traF* Ap^r	29
pGZ119EH (50)	Cm^r (vector for Tra 2 region cloning)	29
pJF119EH (55)	Ap^r (vector for traF and *traG* cloning)	11

[a] SDS, sodium dodecyl sulfate.
[b] wt, wild type.
[c] pLM2 is a RP1 derivative carrying amber mutations in the *bla* and *tetA* genes.
[d] pML123, pGZ119EHΩ[RP4 18,841 to 30,042 bp].
[e] pWP471, pJF119EHΩ[RP4 45,909 to 46,577 bp].
[f] pML100, pJF119EHΩ[RP4 45,870 to 48,933 bp].

TABLE 2. Plating efficiency of PRD1 and the number of receptors on cells with different IncP plasmid Tra region derivatives

Strain	PRD1 titer[a]	Approx no. of PRD1 receptors per cell[b]
JE2571	0	0
JE2571(RP4)	1.2×10^{11}	25
JE2571(pLM2)	1.6×10^{11}	25
JE2571(pML123)	0	0
JE2571(pML123, pWP471)	1.8×10^{11}	80
JE2571(pML123, pML100)	2.0×10^{11}	25
DS88 (pLM2)	9.5×10^{11}	60

[a] The plating efficiency was determined with phage stock grown on DS88 cells. The result was essentially the same when the phage stock grown on *E. coli* cells was used.

[b] The number was obtained from the MOI-dependent saturation, or extrapolation of the saturation in the case of JE2571(pML123, pWP471), as shown in Fig. 2.

RESULTS

Overexpression of the Mpf complex in *E. coli* results in up to about 80 PRD1 receptors on the cell surface. As the Mpf system of IncP plasmids is known to encode the receptor for phage PRD1, we first tested the phage sensitivity of cells overexpressing the Mpf system versus that of control strains lacking part of Mpf or carrying the parental plasmids RP4 or pLM2 (Table 2). Although PRD1 is a broad-host-range phage, the efficiency of plating (EOP) is species specific. This is indicated by the EOP of *E. coli* JE2571(pLM2), which is only about 17% of that obtained with *Salmonella typhimurium* DS88. Cells harboring a plasmid containing the Tra2 core region (pML123) were fully resistant to PRD1. However, the addition of a second plasmid containing the *traF* gene from the Tra1 region (JE2571[pML123, pWP471]) restored full sensitivity to PRD1, the EOP being at the same level as for cells containing the original plasmid (RP4 or pLM2).

The results of the MOI-dependent phage binding experiments (receptor saturation experiments; Fig. 2) with the Tra variants are in agreement with those of the plating efficiency experiments. Even at the highest MOI (300) tested, JE2571 cells or those carrying the Tra2 core region only did not adsorb any phages. The positive control DS88 cells bound somewhat less than 60 phages/cell, which is in good agreement with previous results (23). *E. coli* cells with the parental plasmids (RP4 or pLM2) or those with plasmids containing the Tra2 core region plus the *traF* and *traG* genes (JE2571[pML123, pML100]) expressed about 25 receptors on their cell surfaces. This is also in agreement with previous *E. coli* results (23). However, even at the highest MOI used, cells containing the Tra2 core region together with *traF* (JE2571[pML123, pWP471]) did not reach saturation. Extrapolation suggested that there were some 80 receptors per cell. The amount of TraF specified by pWP471 is about fivefold higher than that in constructs carrying pML100 due to the replacement of the original translation initiation region of the RP4 *traF* gene by that of bacteriophage T7 gene 10. However, the other Mpf components originate from plasmid pML123, which is used in all these experiments.

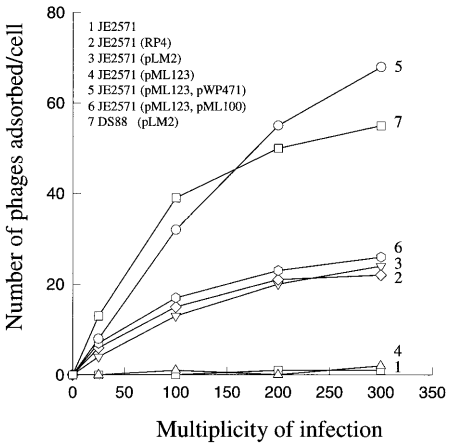

FIG. 2. MOI-dependent adsorption of PRD1 particles on the surfaces of cells harboring plasmids containing a variety of IncP-type Tra region genes.

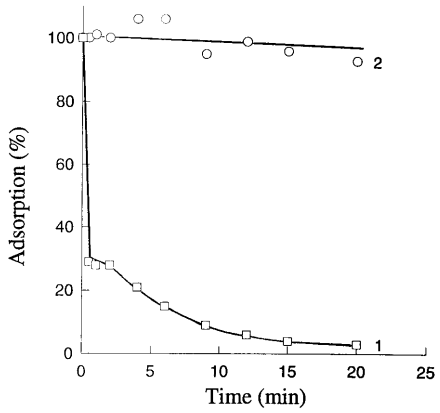

FIG. 3. Effect of arsenate incubation time on PRD1 adsorption for *S. typhimurium* DS88 cells. The experiments were carried out in 50 mM Tris-HCl (pH 8.0) containing 0.1% glucose. The cell concentration was 3×10^9 cells/ml, and the cells were preincubated at room temperature for 10 min before phage or arsenate additions. (Curve 1) Sodium arsenate (20 mM) was added first, and phage particles (MOI, 4) were added after the indicated time periods; (curve 2) phage particles were added first, and arsenate was added after the indicated time periods. The infection mixtures were incubated for an additional 10 min after the second addition before the number of adsorbed phages was assayed. The results were normalized to nontreated cells (100%).

The dynamics of receptor complex disassembly was investigated by using energy-depleted cells (7) and the phage adsorption assay. Twenty millimolar arsenate, drastically decreasing the concentration of intracellular ATP, reduces PRD1 adsorption very effectively (7). The adsorption kinetics analysis showed a 70% reduction in the number of phage particles bound during the first 30 s after arsenate addition (Fig. 3, curve 1). This rapid reduction was followed by a slower one, which continued for 15 min. However, the reduction of the intracellular ATP content (addition of arsenate) had no effect on the number of phage particles adsorbed if arsenate was added after the phage (Fig. 3, curve 2).

The presence of the Mpf complex affects TPP^+ uptake. The use of TPP^+ for $\Delta\Psi$ measurements requires OM permeabilization, which is normally achieved by Tris-EDTA treatment (3, 20). However, this treatment led to the partial lysis of JE2571 cells, although the standard control cells of strain AN180 stayed intact. For this reason TPP^+ uptake by different subassemblies of DNA transfer complex-containing cells was studied in 50 mM Tris-HCl buffer, pH 8.0. The hypotonic Tris medium increased the OM permeability to TPP^+, but the degree of increase depended on the *tra* gene content of the cells. The parent JE2571 strain and the strains with the original plasmids (RP4 or pLM2) had a low initial TPP^+ uptake, which was greatly enhanced by the addition of EDTA (Fig. 4). However, each strain which contained a plasmid with the Tra2 system

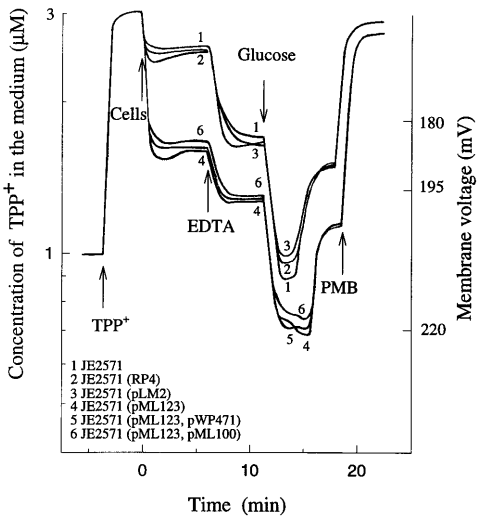

FIG. 4. Influx of TPP^+ into cells expressing various Tra region genes. The experiments were carried out at 37°C in 50 mM Tris-HCl, pH 8.0, initially containing 1 μM TPP^+. TPP^+ was added to obtain a 3 μM final concentration in the medium; *E. coli* cells were added to a final concentration of 1.5×10^9 cells/ml; and EDTA, glucose, and PMB were added to final concentrations of 0.5 mM, 0.1%, and 150 μg/ml, respectively.

(pML123) showed higher initial TPP^+ uptake, which was also enhanced by EDTA. The addition of glucose to the bacterial suspension induced an immediate accumulation of TPP^+, but this uptake was followed by a rapid efflux of additionally accumulated TPP^+ (Fig. 4). A second addition of glucose had no effect on TPP^+ accumulation (data not shown). Polycationic antibiotic polymyxin B (PMB) induced a quantitative release of accumulated TPP^+ (Fig. 4).

The TPP^+ uptake experiments clearly distinguished between strains having plasmids with the complete Tra system and those which contained only the Mpf genes or a subset of them. It was difficult to detect any differences in accumulation of TPP^+ by the cells within each group. When a high level of permeability of the OM to TPP^+ is achieved by adding EDTA, it is possible to calculate the values of $\Delta\Psi$ according to the distribution of this cation between cell cytosol and the surrounding medium. In the absence of glucose but in the presence of EDTA, the plasmid-less JE2571 cells and those containing RP4 or pLM2 plasmids displayed about 20% lower accumulation of TPP^+ than the cells containing plasmid pML123. This corresponds to a difference in $\Delta\Psi$ of about 15 mV (Fig. 4).

OM permeability is transfer complex specific. The ease of using antibiotic resistance markers for selection has made them widely used in gene manipulations. However, in some situations resistance genes (9, 13) as well as high-copy-number plasmids (49) can interfere with the analysis of studied phenomena. In addition, growth of cells in antibiotic-containing media results in activation of the chromosome-coded multiple antibiotic resistance system (12). For this reason the corresponding strain with the vector plasmid containing the same antibiotic resistance marker was used as a control.

To study the nature of the increased TPP^+ uptake by pML123-containing cells, the accumulation of this cation was measured at two temperatures (25 and 37°C). The equilibrium amount of TPP^+ accumulated depends on at least four factors: (i) $\Delta\Psi$, (ii) permeability of the OM to TPP^+, (iii) efficiency of lipophilic cation-extruding pumps (41), and (iv) the cell surface charge. In the case of isogenic JE2571(pGZ119EH) and JE2571(pML123) strains, for which the final levels of TPP^+ accumulation in the presence of EDTA are similar (Fig. 5), the ratio of the amount of TPP^+ accumulated by the cells before the addition of EDTA to the amount of TPP^+ accumulated by the cells in the presence of EDTA is an indicator of the initial OM permeability to TPP^+. At the lower temperature, the initial TPP^+ uptake by the incomplete Tra region-containing cells (as shown for JE2571[pML123] in Fig. 5A) was close to that of strain KO1489, which is known to be highly permeable to TPP^+ (15). Qualitatively, the modes of TPP^+ uptake by pML123-containing strains were similar at both temperatures, although in the case of control strain KO1489 or vector plasmid-containing JE2571(pGZ119EH) cells at 37°C, the initial phase of TPP^+ uptake was much more intensive. The behavior of the cells containing RP4 or pLM2 plasmids (as shown for JE2571[RP4] in Fig. 5) was similar to that of the control plasmid-less JE2571 strain (data not shown).

The difference in OM permeability was also analyzed by studying the effects of ionophoric antibiotics on the accumulation of TPP^+. In the presence of EDTA, the OMs of all of the cells studied were permeable to NG (a K^+-to-H^+ exchanger), which increased $\Delta\Psi$ to its maximal level. Gramicidin D (GD; a channel former) induced considerable TPP^+ efflux only from control KO1489 cells and cells containing plasmid pML123 and only at 37°C (Fig. 5B). The amount of TPP^+ released after the addition of GD depends on (i) the permeability of the OM to GD and (ii) the level of $\Delta\Psi$. If the levels of membrane voltage are similar, as in the case of JE2571(pGZ119EH) and JE2571(pML123) cells (Fig. 5), the ratio of the amount of TPP^+ released from the cells after the addition of GD to the amount of TPP^+ released from the cells after the addition of GD and PMB can be also used as an indicator of OM permeability. The low efficiency of GD on the vector plasmid pGZ119EH-containing cells also indicates that the presence of the Mpf proteins increases OM permeability. PMB induces an additional increase in OM permeability to GD and forms ion-permeable pores in the bacterial PM itself (8). These effects lead to a complete depolarization of the PM (Fig. 5).

These results suggest that the increased permeability of the OM to lipophilic compounds is a result of the presence of the Tra2 complex in the cell. However, the higher $\Delta\Psi$ of pML123-containing cells is probably caused by systems encoded outside the Tra2 region. We also detected that the amount of TPP^+ irreversibly bound to chloramphenicol-resistant cells was higher than the amounts irreversibly bound to cells of other strains studied (Fig. 5).

The presence of the Mpf system decreases intracellular K^+ content. The effect of the Tra system on the functioning of the PM was studied by analyzing the K^+ content of the cells. The cells were not able to maintain a high intracellular K^+ concentration in 50 mM Tris buffer (Fig. 6A). The addition of EDTA accelerated K^+ leakage. Glucose stabilized the intracellular potassium ion concentration except when pML123 was present, in which case glucose stimulated K^+ leakage (shown for JE2571[pML123]). At 25°C, the leakage-stimulating effect of EDTA was weaker but the glucose-induced efflux of K^+ from Mpf complex-containing cells was stronger (data not shown). NG addition induced K^+ efflux from the JE2571 strains studied regardless of their plasmid content. GD had little effect on the K^+ content, but after addition of PMB the cumulative effect of GD, NG, and PMB dissipated the K^+ gradient across the PM, thereby equilibrating K^+ between the cytosol and the external milieu.

EDTA did not induce K^+ leakage from the cells suspended in phosphate medium (Fig. 6B), and the addition of glucose

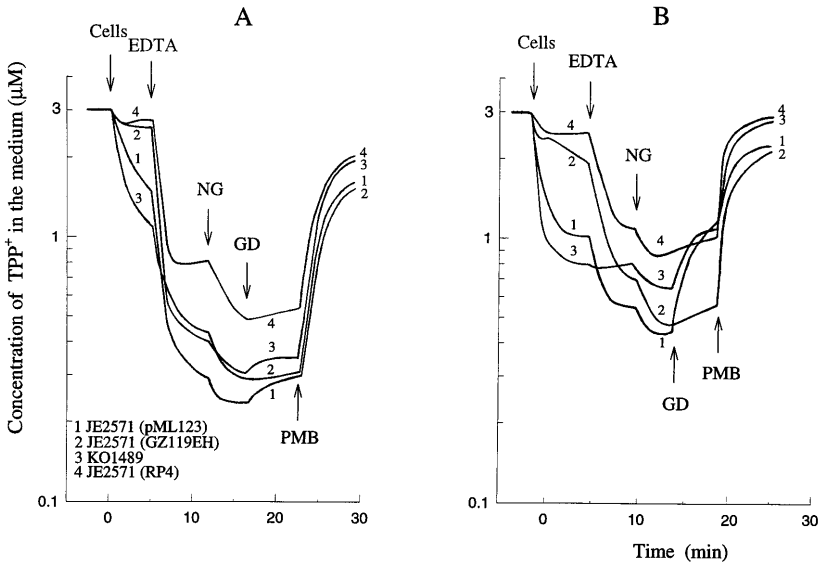

FIG. 5. Effect of temperature on TPP^+ uptake. The experiments were carried out in 50 mM Tris-HCl, pH 8.0, containing 3 μM TPP^+ and 3×10^9 cells/ml. EDTA was added to obtain a 0.5 mM final concentration. NG, GD, and PMB were added to final concentrations of 2, 4, and 150 μg/ml, respectively. (A) Experiments at 25°C; (B) experiments at 37°C.

caused a considerable uptake of K^+ ions. The glucose-induced K^+ uptake was strongest with JE2571 cells and weakest with cells containing plasmid pML123. Cells containing plasmid RP4 were in between. The initial rates of glucose-induced K^+ uptake were similar in all the cases, but the uptake abruptly stopped 1 to 1.5 min after glucose addition when only the genes coding for the Tra2 complex were present. NG addition caused K^+ efflux, and this efflux was strongest in the case of cells containing plasmid pML123. PMB addition again equilibrated the K^+ ions.

In the presence of glucose the parent JE2571 strain and those containing plasmids RP4, pLM2, or pGZ119 had more than 80% higher intracellular K^+ concentrations than did cells with the Mpf genes only. This difference in K^+ content was lower if EDTA was absent. Cells with plasmid pML123 contained 30 to 40% less K^+ even in the normal growth condition. This is evident as a lower total amount of K^+ is brought into the medium with the addition of pML123-containing cells (shown for JE2571[pML123] in Fig. 6).

The presence of the Mpf system increases cell envelope permeability to ATP. The measurements of ATP-dependent light emission with the luciferin-luciferase system showed that the concentrations of extracellular ATP in cell suspensions were low in the absence of glucose (Fig. 7A). High concentrations of PMB induced an additional leakage of intracellular ATP to the medium and its equilibration between the cytosol and the surrounding medium. In the case of pML123-containing cells (shown for JE2571[pML123, pWP471] in Fig. 7) the

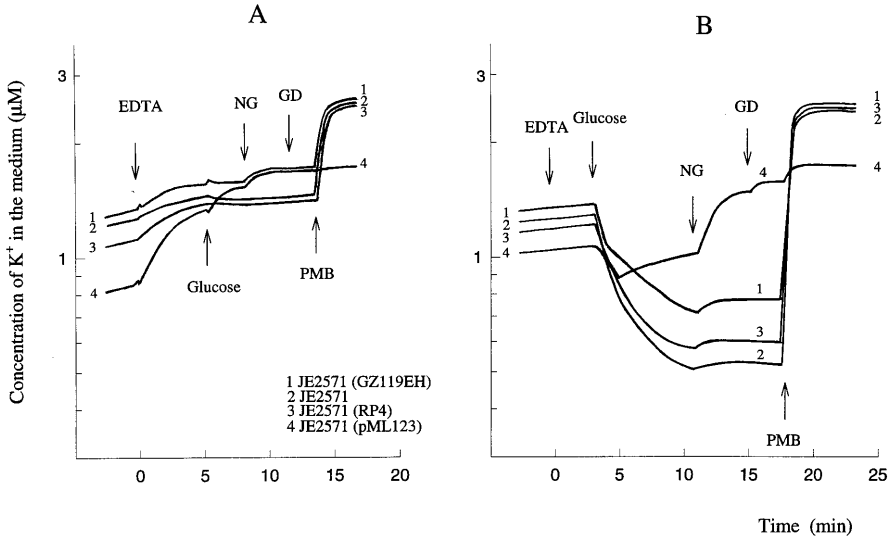

FIG. 6. Effect of glucose and membrane-active antibiotics on K^+ fluxes across the cell envelope. The experiments were carried out in 50 mM Tris (A) or 100 mM sodium phosphate (B), pH 8.0, at 37°C with 3×10^9 cells/ml. Additions: EDTA, glucose, NG, GD, and PMB to final concentrations of 0.5 mM, 0.1%, 2 μg/ml, 4 μg/ml, and 130 μg/ml, respectively.

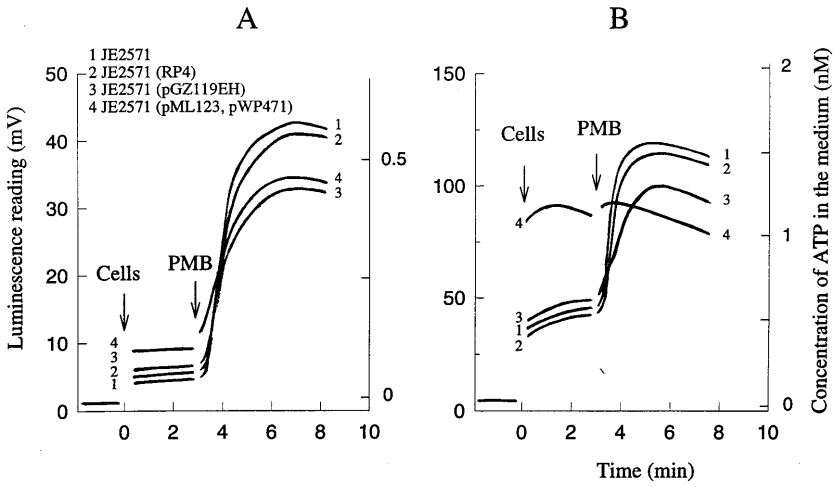

FIG. 7. ATP release from the cells expressing various Tra region genes. A concentration of 3×10^9 cells/ml was preincubated for 8 min at 37°C in 50 mM Tris-HCl, pH 8.0, (A) or the same medium supplemented with 0.5 mM EDTA and 0.1% glucose (B). After preincubation the cells were diluted 20-fold by the same medium containing 1/4 (vol/vol) ATP monitoring reagent, and the intensity of luminescence was measured at room temperature. PMB was added to a final concentration of 500 μg/ml.

initial as well as the final ATP concentration in the medium was somewhat higher than in the case of control cells containing vector plasmid pGZ119EH.

The initial concentration of ATP in the medium was considerably higher if the cells were preincubated with EDTA and glucose. The addition of PMB induced an additional increase of the luminescence (Fig. 7B). However, PMB had very little effect on the intensity of luminescence in the case of JE2571 (pML123, pWP471) and other plasmid pML123-containing cells, indicating that the envelopes of these cells were highly permeable to ATP in these conditions. In the absence of either glucose or EDTA the permeability to ATP of Tra2-containing cells was considerably lower (data not shown).

DISCUSSION

We investigated the effects of the IncP Mpf complex, a DNA transport and PRD1 receptor structure, on $\Delta\Psi$, K^+, and ATP

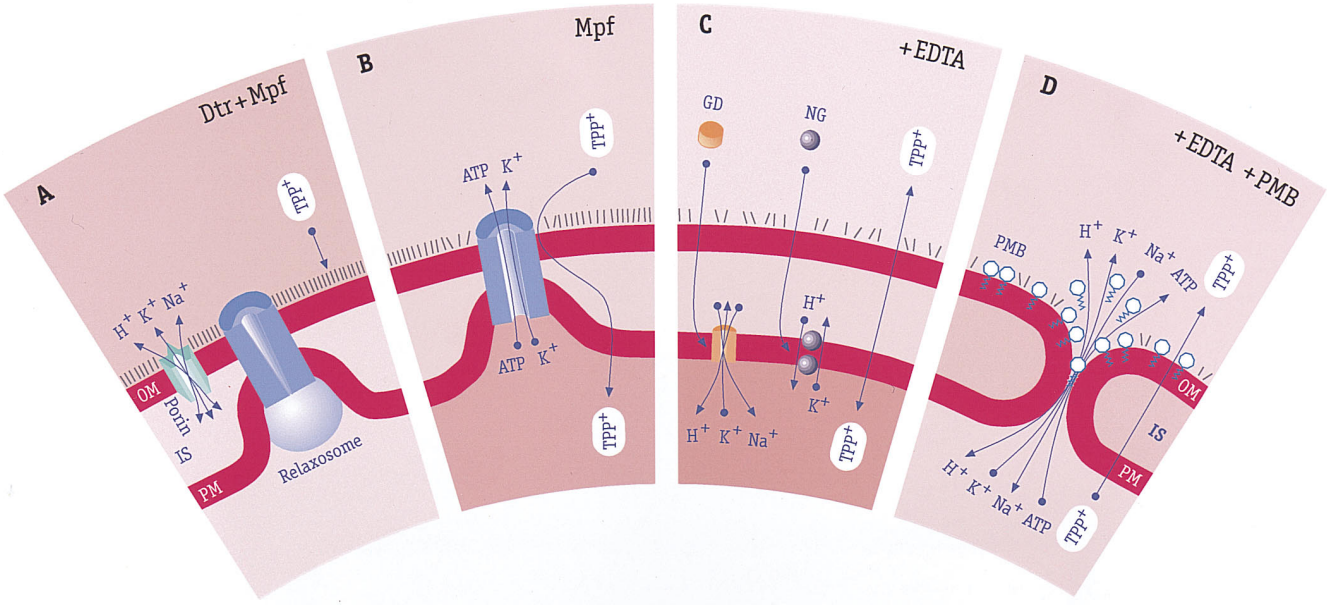

FIG. 8. Proposed routes of ion and ATP fluxes through the envelope of *E. coli*. (A) Cells with an intact IncP-encoded DNA transport system. LPS molecules (only simplified polysaccharide chains are shown) exist in an ordered quasicrystalline arrangement. Hydrophilic, but not lipophilic, ions and ATP cross the OM through the porins. TPP^+ and ionophoric antibiotics cannot penetrate the OM. (B) Cells with the Mpf complex only. The OM has an increased permeability to TPP^+. This lipophilic cation is able to accumulate in the cytosol at levels that depend on the membrane voltage. The cells have an increased permeability to K^+ and ATP. (C) EDTA-treated cellular envelope. Due to removal of divalent cations, some of the LPS and, therefore, the OM barrier to lipophilic compounds is lost, but the PM remains intact. TPP^+ freely penetrates the OM and accumulates in the cytosol. Following NG-induced K^+-to-H^+ exchange, $\Delta\Psi$ and, correspondingly, TPP^+ accumulation in the cytosol attain a maximum. GD induces the depolarization of the PM, the extent of which depends on the permeability of the OM to this antibiotic. (D) EDTA-plus-PMB-treated cellular envelope. PMB binds to the cellular envelope and induces pores at zones of adhesion. The OM permeability barrier is completely lost, the PM is depolarized, and gradients of ATP and K^+ on the PM are abolished.

levels in *E. coli*. The complete Mpf system (Tra2 plus *traF*) is required and sufficient for phage adsorption and DNA injection. The removal of the *traF* gene or any one of the Tra2 core region genes inactivates this function (18). This result complicates the search for the Tra2 component that functions as the receptor protein because each of the Mpf complex components is a potential candidate. However, the number of possible candidates is likely to be reduced by the fact that the receptor protein must be accessible from the outside. Sequence alignment indicates that proteins TrbC, -G, -H, and -L fulfill some requirements for OM localization (27). In addition, the observation that each Tra2 core component is essential for PRD1 propagation suggests that a complex structure of 12 protein species is involved in the transport of the receptor protein to the OM or in receptor assembly if the receptor consists of a multiprotein complex. The disappearance of the receptor after arsenate addition (Fig. 3) indicates that energy in the form of ATP is necessary to keep the receptor complex assembled on the cell surface.

It is known that changes in OM protein composition can increase its permeability to lipophilic compounds (for reviews, see references 19 and 48). The *sfrB* mutation of *E. coli* K-12 affects the expression of the *tra* cistrons of the F plasmid as well as lipopolysaccharide (LPS) synthesis. Besides a decrease in F pilus synthesis, DNA transfer frequency, and surface exclusion, this mutation leads also to increased sensitivity to some antibiotics and dyes (48). Incorrect assembly of the Mpf gene products in the absence of the Dtr system could explain the greater permeability of the cells containing the incomplete Tra system. However, the Mpf complexes of the "permeable" cells studied here are fully functional in DNA transfer (18, 29) and phage adsorption. This data confirms that the differences detected are due to an active Mpf complex. The measurements of TPP^+ could not distinguish between strains for which the only difference in the Mpf complex was the presence or absence of the TraF protein, indicating that the basal structures (structural frames) are the same in both cases.

The electrochemical measurements showed that the presence of pML123, which encodes a predicted proteinaceous structure in the cell envelope, made the OM more permeable to lipophilic compounds and increased the permeability of the PM to potassium ions and ATP but did not depolarize the PM. The suppression of the permeability caused by the addition of Dtr genes is an intriguing observation. The Dtr gene products, which are located in the cytosol and possibly in the PM, may be proteins that bind the relaxosome to the Mpf structure facing the cytosolic side of the PM. These proteins alone or in combination with relaxosome components could play a role in regulation of the permeability of Mpf complex-induced pathways. Figure 8 depicts the events thought to take place in the cell envelope in the system studied here (for details see the figure legend).

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