STUDIES ON DNA TRANSPORT DURING BACTERIAL CONJUGATION. ROLE OF PROTONMOTIVE FORCE-GENERATING H^+-ATPase AND RESPIRATORY CHAIN

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1. Introduction

Recently it has been hypothesized [1] that transport of nucleic acid into a recipient bacterial cell during genetic transformation, transfection and bacterial conjugation is driven by a protonmotive force. According to chemiosmotic theory [2] this force is generated by two independent enzymic systems: respiratory chain and/or H^+-ATPase. Therefore, the use of a F^- parent which is devoid of H^+-ATPase activity [3] and membrane potential generation [4] has enabled us to examine the role of H^+-ATPase and respiratory chain in the process of DNA transfer. The experimental material presented in this report indicates that the genetic recombination depends on the generation of a protonmotive force by the above mentioned enzymic systems of the recipient cell.

2. Materials and methods

The F^- parents used were *Escherichia coli* K12 strain AN 180 (F^-, *arg*$^-$, *thi*$^-$, *mtl*$^-$, *xyl*$^-$, *str*^{r}) and isogenic strain AN 120 (unc A 401), generous gifts of Professor F. Gibson, Australian National University. The Hfr parent used was strain K10 (*Hfr, str*^{s}) kindly supplied by Dr V. Sukhodolec, Institute of Genetics, Moscow. A minimal salt medium was that of Curtiss [5]. This medium was supplemented with 0.2% casein hydrolysate, 0.1% yeast extract and 0.4% glucose. Cultures of AN strains were grown at 37°C up to 10^9 cells/ml and harvested as described earlier [4]. Cultures of K10 strain were grown in the same medium at 37°C without agitation, up to 5×10^8 cells/ml. Mating was carried out for 1.5 h at 37°C as described by Curtiss et al. [6] in the minimal salt medium (pH 6.3) supplemented with 0.2% casein hydrolysate and 0.4% glucose. Mating pairs were disrupted by agitation on a mixer, diluted with minimal salt buffer [16] and placed at 37°C on solid medium consisting of 1.5% agar, minimal salt medium, 0.4% glucose and streptomycin sulfate (1 mg/ml). For mating under anaerobic conditions 2.5 ml of male and female cell suspensions were mixed in Tunberg-tubes immersed in a water bath at 37°C and the air removed with a vacuum pump. Mating under aerobic conditions was done in 5 ml mating mixture spread on the bottom of a 250 ml conical flask. Respiration was measured with a Clark-type oxygen electrode. Respiratory activity was expressed in nmoles of oxygen reduced/min/mg of dry cell weight. The washed cells were disrupted using a UZDN-1 sonifier and prepared according to Futai et al. [7]. The rate of the ATPase reaction was followed by the change in pH of the reaction mixture [8]. ATPase activity was expressed in nmoles of ATP hydrolysed/min/mg of protein. The uptake of TB^- by membrane particles was measured by means of phospholipid films, as described earlier [9,10].

Abbreviations: H^+-ATPase, H^+ ion translocating, Mg^{2+}, + Ca^{2+}-dependent ATPase (EC 3.6.1.3); TB^-, tetraphenylboron; CCCP, carbonylcyanide 2,4-dichlorophenylhydrazone.

3. Results

The respiratory activity of the strains used in our experiments did not differ significantly and varied in

the range 60–90 units. The experiments with the 'inside-out' membrane particles confirmed the earlier published evidence [3] that a membrane of AN 120 strain is essentially devoid of the H^+-ATPase activity. In our experiments the membrane particles from AN 180 and K10 strains manifested the H^+-ATPase activity close to 600 units while the H^+-ATPase activity of membrane of strain AN 120 was equal to 4 units.

Several authors [11,12] have shown that ATP hydrolysis is coupled with the movement of H^+ ions across the membrane of *E. coli* cell particles. Figure 1 depicts membrane potential generation in 'inside-out' membrane particles prepared from the cells of above listed AN strains. A membrane potential has been detected by the penetrating anion method. Earlier it was shown [13] that accumulation of penetrating anions inside membrane particles reflects generation of an electrical charge imbalance across the membrane of positive sign inside the particles.

As seen in fig.1A, the addition of NADH to the suspension of strain AN 180 membrane particles previously equilibrated with penetrating anions induces the uptake of TB^-. The inhibition of respiration by cyanide reverses the NADH-induced uptake of TB^-. Injection of ATP into the same suspension causes TB^-accumulation inside the particles. Treatment of the particles with the uncoupler CCCP induces TB^- loss from the particles and brings the TB^- concentration to the initial level.

As can be seen in fig.1B, addition of ATP has no effect on the TB^- concentration in the suspension of strain AN 120 membrane particles. The TB^- uptake is initiated by NADH addition and reversed by an

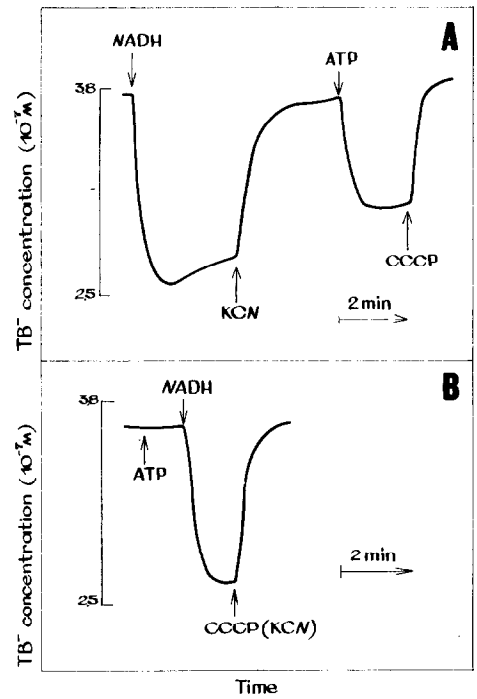

Fig.1. Comparison of the energy-linked TB^- uptake by 'inside-out' membrane particles of AN 180 (A) and AN 120 (B) strains. Incubation mixture consisted of 0.02 M Tris–H_2SO_4 (pH 8.0), 2 mM $MgSO_4$, 1 mM $CaCl_2$ and 4.6×10^{-7} M TB^-. Incubation mixture was supplemented with 0.3 mg/ml of strain AN 180 membrane particles (see expt. A) or with 0.2 mg/ml of strain AN 120 membrane particles (see expt. B). Additions: 1 mM NADH, 10 mM KCN, 1×10^{-6} M CCCP and 1 mM ATP.

Table 1
Effect of protonmotive force generation in the F^- parent on recombinant production

Hfr parent (donor)	F^- parent (recipient)	Mating conditions	Protonmotive force in the recipient cell during mating	Frequency of *arg*$^+$, *thi*$^+$ recombinants	
				Actual	Relative
K10	AN 180	Aerobic	Generated	5×10^{-4}	100
K10	AN 180	Anaerobic	Generated	3×10^{-5}	6
K10	AN 120 (H^+-ATPase$^-$)	Aerobic	Generated	3×10^{-5}	6
K10	AN 120 (H^+-ATPase$^-$)	Anaerobic	Not generated	$<10^{-7}$	<0.02
K10	AN 120 (H^+-ATPase$^-$)	Anaerobic → aerobic[a]	Generated	4×10^{-5}	8

[a]Mating mixture was incubated for 1.5 h anaerobically, then diluted 100 times with aerobic medium and incubated for a further 1.5 h aerobically. All other experiments were done as described in Materials and methods.

uncoupler of oxidative phosphorylation or by the respiratory inhibitor cyanide.

These results give direct support to the earlier conclusion [4] that in cells of strain AN 120 a protonmotive force is solely generated by the respiratory chain while in cells of strain AN 180 both the H^+-ATPase and the respiratory chain create a gradient of H^+ ion electrochemical potentials.

The data presented in table 1 demonstrate that the frequency of genetic recombination depends greatly on the generation of a protonmotive force in the recipient cell. Comparison of the data in lines 1 and 2 (table 1) indicates that a F^- parent containing an intact H^+-ATPase and respiratory chain yields recombinants under both aerobic and anaerobic mating conditions. It has been shown [14] that lowering the temperature results in the loss of F pili by donor cells. Therefore, a decrease in recombinant production under anaerobic conditions could be due to the drop in the temperature during air evacuation.

The data in line 3 (table 1) show that under aerobic conditions a F^- parent devoid of protonmotive force-generating H^+-ATPase acts as a genetic information recipient. Under anaerobic conditions the yield of recombinants is greatly decreased (see line 4, table 1). This effect can not be explained by the damage to F pili. The data in line 5 (table 1) indicate that dilution of an anaerobic mating mixture with aerobic mating medium results in the same frequency of recombination as mating under aerobic conditions (compare lines 3 and 5, table 1). Control experiments have shown that dilution of parent cultures before mixing, reduced recombinant yield. This, it can be concluded that under anaerobic conditions specific pairs between donor and recipient cells were formed.

4. Discussion

Curtiss et al. [6] subdivided bacterial conjugation into five steps: '(i) formation of specific pairs between donor and recipient cells, (ii) conversion of specific pairs to effective pairs or conjugation tube formation, (iii) chromosome mobilization or the initial events in the donor cell for the conversion of a circular chromosome into a chromosome capable of being sequentially transferred, (iv) chromosome transfer and (v) integration of the recipient chromosome to produce recombinants'.

A protonmotive force in the recipient cell seems not to be involved in step (i) since an F^- parent devoid of H^+-ATPase forms mating pairs under anaerobic conditions. Furthermore, it has been reported [15] that specific pair formation is an energy-independent process. The events mentioned in the step (iii) can not have contributed to the observed effects since the donor cells were able to donate their chromosomes under aerobic as well as under anaerobic conditions. It has been shown earlier [17] that addition of O_2 or NO_3^- to an anaerobic culture of ATPase-deficient cells leads to restoration of DNA replication blocked under anaerobic conditions. The experiments reported above indicate that recombinant production can be restored by the aeration of an anaerobic suspension of mating pairs. The aeration of the suspension of separated parents did not induce the production of recombinants. This argues against the suggestion that the inhibition reported above of recombinant production is due to the anaerobic block in DNA replication or integration. Therefore, it seems possible to conclude that a protonmotive force in recipient cell is involved in steps (ii) and/or (iv).

The mechanism of protonmotive force involvement in DNA transfer needs further study.

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