Hypothesis

NUCLEIC ACID TRANSPORT DRIVEN BY ION GRADIENT ACROSS CELL MEMBRANE

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1. Introduction

Molecules of nucleic acids are transported across the membrane into the recipient cell at an early stage of genetic transformation, transfection, viral infection and conjugation. Bacterial as well as plant and animal cells are able to bind nucleic acids and take them up. The mechanism of DNA transport is one of the most intriguing, yet unexplored problem of vectorial metabolism.

During the early stages of genetic transformation cells of *Bacillus* and *Diplococcus* adsorb DNA molecules from the surrounding medium and convert them to double-stranded fragments (reviewed [1]). In a few minutes the double-stranded DNA fragments are converted into single-stranded DNA fragments which are linearly transported into host cell cytoplasm and form there a donor–recipient DNA complex. On the other hand, the appearance of the single-stranded DNA fragments is probably no necessarily coupled with DNA transport into bacterial cells. The experimental evidence available at present [1] indicates that single-stranded DNA does not appear during the early stage of *Haemophilus influenzae* transformation. Furthermore, direct proof that either strand of a DNA molecule can transform has been obtained in experiments with *Bacillus subtilis* DNA. The two strands of this DNA have been separated and each of them transformed *B. subtilis* cells to approximately the same extent [2,3].

The ability to take up 'naked' DNA molecules is not unique to the Gram-positive bacteria. Destruction of the cell wall of Gram-negative *Escherichia coli* cells produces osmotically-sensitive spheroplasts which are competent to take up DNA molecules [4]. The calcium-induced uptake of DNA by intact gram-negative cells was discovered by Mandel and Higa [5]. DNA is introduced into intact cells in two steps:

(1) The cells are incubated with calcium salts and DNA at 0°C;
(2) The suspension is then warmed to 37°C.

Viruses are initially adsorbed to receptors in the outer cell wall and then transfer their nucleic acid molecules without damage of these molecules during transport. Large T-even bacteriophages possess a contractile protein tail [6], but proteins of small bacterial viruses manifest no contractile ability (reviewed [7]).

During the early stage of bacterial conjugation a donor cell is adsorbed by its pili to the recipient cell and donor DNA cleavage and membrane structure rearrangement are initiated. Thereafter, the single-stranded part of the donor DNA molecule enters the recipient cell by its 5′-end [8,9].

To obtain efficient uptake of nucleic acids plant cells are usually converted into protoplasts [10]. The basic polypeptide polyornithine and Zn^{2+} stimulate the uptake of nucleic acids by the protoplasts. Polyornithine also enhances the uptake of exogenous DNA by animal cells, while DEAE-dextran, $CaCl_2$, latex spheres, spermine, polylysine and polyarginine are less effective [10].

When considering possible mechanisms of nucleic acid transport across cell membrane one should note several features of the process:

(1) DNA has to penetrate a cytoplasmic membrane characterized by transmembranous imbalance of electrostatic potentials. The value of the imbalance reaches 140 mV, negative inside the cell (reviewed [11,12]).
(2) DNA transport through the bacterial membrane is accomplished linearly [13–15]. Thus the part of DNA molecule transversing the membrane is linear, but segments outside the membrane might have a compact conformation.
(3) The protonophorous uncouplers of oxidative

phosphorylation and the ionophorous antibiotics nigericin and valinomycin have an inhibitory effect on the early stages of genetic transformation of Gram-positive bacteria [16–20]. The inhibitory effect of uncouplers on the transformation of *B. subtilis* is correlated with their effect on the membrane potential of the cell [20]. The inhibition of *B. subtilis* transformation by uncouplers occurs despite the fact that the phosphorylation potential of the glycolyzing host cell remains at a constant level [20].

(4) The sensitivity of DNA transport during bacterial conjugation towards oxidative phosphorylation uncouplers as well as the dependence of DNA transport on the energy-yielding activity of the recipient cell have been demonstrated [21–23]. The maintenance of a high-energy membrane state by recipient *E. coli* cells produces irreversible absorption of phages T1 and ϕ 80 [24]. On the other hand, phage T4 containing a contractile protein tail is irreversibly adsorbed by de-energized *E. coli* cells. Phage T4 adsorbed to de-energized cells do not form an infective complex [25], indicating an inhibition of phage DNA entry into the cell cytoplasm.

These findings show that the process of external nucleic acid entry, at least into bacterial cells, if not also into plant and animal cells, depends on the high-energy state of the recipient cell membrane. The high-energy membrane state of a bacterial cell is the gradient of H^+ electrochemical potential across the membrane [11,12]. The gradient consists of an electrical term (a membrane potential) and a chemical term (a transmembranous pH gradient).

2. Explanations of nucleic acid entry

Several concepts have been proposed to explain nucleic acid entry, but none of them has been proven. Most are contradictory and only aim at explaining a strictly specified process of nucleic acid entry such as the early stages of genetic transformation or viral infection, but no unified concept covering all aspects has been formulated. Here I shall discuss some of these concepts.

(1) A replicative mechanism of DNA transport has been suggested to explain DNA transport during the early stages of bacterial conjugation [26]. According to this concept, the entering strand comes from donor DNA replicating asymmetrically to produce a single-stranded product. Similar mechanisms have been suggested for the early stages of genetic transformation, the only difference being that DNA transport proceeds because of the replication of the entering single-stranded DNA in the host cell [27].

The demonstration of DNA transfer in the absence of DNA replication in the recipient or donor cell [28,29] fits poorly with the replicative mechanism of DNA transport. Furthermore, no relationship has been observed between DNA replication and the integration of entering DNA into the host cell chromosome [30–32].

(2) A mechanism of DNA transport was proposed by Lacks [33,34] for the early stage of bacterial genetic transformation. He suggested that single-stranded DNA transport is catalyzed by membrane-bound DNase which forms a multimeric protein cylinder that spans the membrane and provides an aqueous channel for the passage of single DNA strands into the cell. The entry begins with the passage of a single strand through the channel, in which the DNase clips away the opposite strand. The hydrolysis of this strand may provide the motive force for DNA entry.

The uptake of double-stranded DNA by *H. influenzae* cells [35,36] (but also see [37]) limits application of Lacks' mechanism. This mechanism also provides no explanation for the ability of *Diplococcus, Haemophilus* and *Bacillus* species to take up single-stranded DNA [38–41]. Despite the appearance of single-stranded intermediate products during the early stages of *Diplococcus pneumoniae* and *B. subtilis* transformation [34,42], these observations provide no convincing evidence for DNase-catalyzed DNA transport. The experiments in [43] with *D. pneumoniae* have shown that loss of DNA into the medium and DNA degradation are limited in the presence of Ca^{2+} alone, while the DNA uptake and genetic transformation reaches the maximum attainable levels. Furthermore, Lacks' mechanism of DNA transport provides no explanation for the inhibitory effect of uncouplers on the early stages of genetic transformation.

(3) The mechanism of bacterial genetic transformation formulated by Akrigg et al. [44] suggests that lysis of sections of the cell wall is followed by mesosomal vesicles protruding through the resulting holes in the cell wall and binding DNA

molecules from the surrounding medium. This binding brings DNA close to the host cell DNA replication point which, as postulated [44], is localized in the mesosomes. According to this hypothesis, the energy required for DNA transport is provided by respiratory chain enzymes.

The presence of some unknown energy-dependent mechanism of DNA transport was also suggested in [45]. The idea that energy is needed for DNA transport during bacterial conjugation was formulated in [46].

(4) A DNA 'injection' mechanism has been proposed [47,48] for the early stage of bacteriophage infection. This concept is based on the ability of several phages to release their DNA into the surrounding medium upon contact with isolated cell wall receptors [49].

This idea seems to be hardly applicable to small viruses. After phage T5 irreversibly adsorbs onto its specific cell wall receptor, at least part of its DNA is released from the head of the capsid and is attached to the cell surface [50]. This attachment takes place prior to the penetration of the first DNA fragment into the cell cytoplasm. Despite the fact that the entry mechanism of T5 phage DNA is not clear, these observations are hardly compatible with the concept of DNA 'injection'.

(5) Kornberg [51] has hypothesized that the phage coat protein may facilitate passage of the phage nucleic acid through the inner membrane of the cell by forming a membrane pore. According to Kornberg [51], similar protein is also involved in the conjugational DNA transport from donor to recipient cell. Kornberg's hypothesis rests on the fact that both nucleic acid and some coat proteins of certain phages are transported into the cell in approximately equimolar amounts, and that their kinetics of penetration are similar [52–55]. The penetration of phage nucleic acid into the cell may involve the transport of a protein–nucleic acid complex rather than of free nucleic acid [53].

The above concept aims at explaining the kinetic features of nucleic acid transport across the membrane and leaves open the problem of the driving forces of the process.

(6) Two mechanisms have been proposed to explain the ability of animal viruses to infect recipient cells [56]:

1. Viral nucleic acids are taken up by a phagocytic process termed 'viriopexis';
2. An alternative hypothesis assumes that fusion at the surface between the virus envelope and cell membrane is an obligatory requirement for initiating the infective cycle, but no driving force for the transport of the nucleoproteidic virion across cell membrane has been proposed.

Since the suggested concepts are contradictory, there is a need for a unifying concept of nucleic acid transport across the cell membrane. Such a concept has been formulated [57]. It is based on the fact that the cell converts metabolic energy into a gradient of H^+ and/or Na^+ electrochemical potential [11,12] the process being catalyzed by plasma membrane-bound enzymic systems which electrogenically extrude H^+ or Na^+ outward. The concept requires the presence of membrane-bound molecular mechanisms that allow H^+ or Na^+ to return to the cytoplasm while performing DNA transport [57]. Let us formulate here a detailed mechanism of nucleic acid transport.

3. Ion gradient-driven transport of nucleic acid

At physiological pH range the molecule of nucleic acid is a polyanion. Consideration of the ways to maintain electroneutrality during the nucleic acid uptake leads to the conclusion that either cations must be taken up together with the nucleic acid molecule or anions must be extruded from the cell. If electroneutrality of the nucleic acid uptake is preserved, one can expect none if any effect of the membrane potential ($\Delta\psi$) on nucleic acid uptake. This is not the case. The dissipation of $\Delta\psi$ by uncouplers of oxidative phosphorylation or ionophorous antibiotics valinomycin and nigericin leads to the specific inhibition of the nucleic acid uptake thus indicating the involvement of $\Delta\psi$ as well as a transmembrane pH gradient (ΔpH) in the nucleic acid transport [20,25].

On the other hand, several systems of nucleic acid uptake, for example Ca^{2+}-induced DNA transport, manifest great resistance towards uncouplers. These findings prompted the claim that something is wrong with the chemiosmotic mechanism of nucleic acid transport [58].

Another puzzle to be solved is the molecular architecture of the system performing nucleic acid transport across the membrane. The essential feature of

the system must be a transmembrane channel large enough to permit the passage of nucleic acid through the membrane. It seems safe to conclude that the permanent existance of a large channel is incompatible with the experimental evidence indicating the high electrical resistance of the inner membrane of the cell [11,12]. One can conclude that the channel is open only during the interaction of the nucleic acid with the membrane and that the channel is destroyed or closed after nucleic acid uptake is finished.

Some essential features of the system performing nucleic acid uptake can be revealed by considering the proteins involved in nucleic acid transport. The relevant experimental evidence has been obtained during a study of the fate of transforming DNA bound to *Streptococcus sanguinis* [59]. During the early stages of genetic transformation one strand of double-stranded donor DNA was shown degraded while the other strand was complexed with recipient cell material which appears to be protein [59]. Initially the complex formed upon uptake is located outside the inner membrane and can be released from the cell under conditions promoting spheroplast formation. It was suggested [59] that to enter the cell it is necessary to move the complex through the inner membrane. This suggestion agrees quite well with the fact that the single-stranded DNA complexed with the protein(s) has been found inside *S. sanguinis* cells [59]. Despite the isolation of similar complexes from *B. subtilis* [60] and *S. pneumoniae* [61] additional experiments are necessary to prove the ability of the complex to penetrate the membrane. A protein which binds to either single-stranded or double-stranded DNA but not to ribonucleic acid has been isolated by osmotic shock treatment of growing *H. influenzae* cells [62]. Certain mutant strains of *H. influenzae* defective in DNA uptake were found to be deficient in the DNA binding protein, suggesting that the protein participates in the transport of DNA [62]. The penetration of tobacco mosaic virus across a model phospholipid membrane has been observed [63]. Thus, it seems reasonable that under appropriate conditions some nucleoproteidic complexes can penetrate across the cell membrane.

Considerations of the classical observations and recent experiments, discussed above, lead to the chemiosmotic concept of the transport which explains the spectrum of different nucleic acid entry processes. The concept is based on two postulates:

(1) Transmembrane electrochemical potential of ions is the driving force of nucleic acid transport;

(2) The system of DNA transport does not pre-exist in the membrane. It is formed upon interaction of DNA, cofactors and phospholipids and exists until the end of DNA transport.

According to the concept, the uptake of polyanionic molecule of nucleic acid by bacteria or animal cells is coupled with the downhill influx of either H^+ or Na^+. In the medium with high Ca^{2+} concentration the nucleic acid uptake seems to be coupled with the Ca^{2+} influx. The flux of these ions is driven by the membrane potential and/or the transmembrane concentration gradient of the ion.

Differences of H^+, Na^+ and K^+ electrochemical potentials exists on the cytoplasmic membrane of energized bacterial cells [11,12]. Nevertheless, bacterial cells are able to take up DNA in a synthetic Na^+-deficient K^+-rich medium as well as in Na^+-rich K^+-deficient medium [1]. Therefore, it seems unlikely that Na^+ or K^+ gradients are involved in DNA uptake by bacterial cells. On the other hand, data on the early intermediate state of transforming DNA during its uptake by *B. subtilis* indicate that EDTA blocks only the initiation of entry of the DNA molecule into the cell, the succeeding stages of the entry being resistant to the binding of both Ca^{2+} and Mg^{2+} [64].

The gradient of Na^+ electrochemical potential across the cytoplasmic membrane of animal cells is generated by Na^+, K^+-ATPase catalyzing electrogenic Na^+ extrusion from the cell [65]. It seems reasonable to assume that the uptake of nucleic acids by animal cells occurs due to the cotransport with Na^+, but not with H^+. Thus, nucleic acid transport through membranes of bacterial and animal cells seems to be driven by the gradient of H^+ or Na^+ electrochemical potential, correspondingly.

Fig.1 depicts the transport of single-stranded polynucleotide which can be represented by single-stranded DNA or RNA. The mechanism can also be applied to double-stranded DNA transport. In this case the outer surface possesses suitable binding proteins and is devoid of DNase activity.

The key features of the proposed mechanism are as follows:

(1) The polynucleotide forms a positively charged

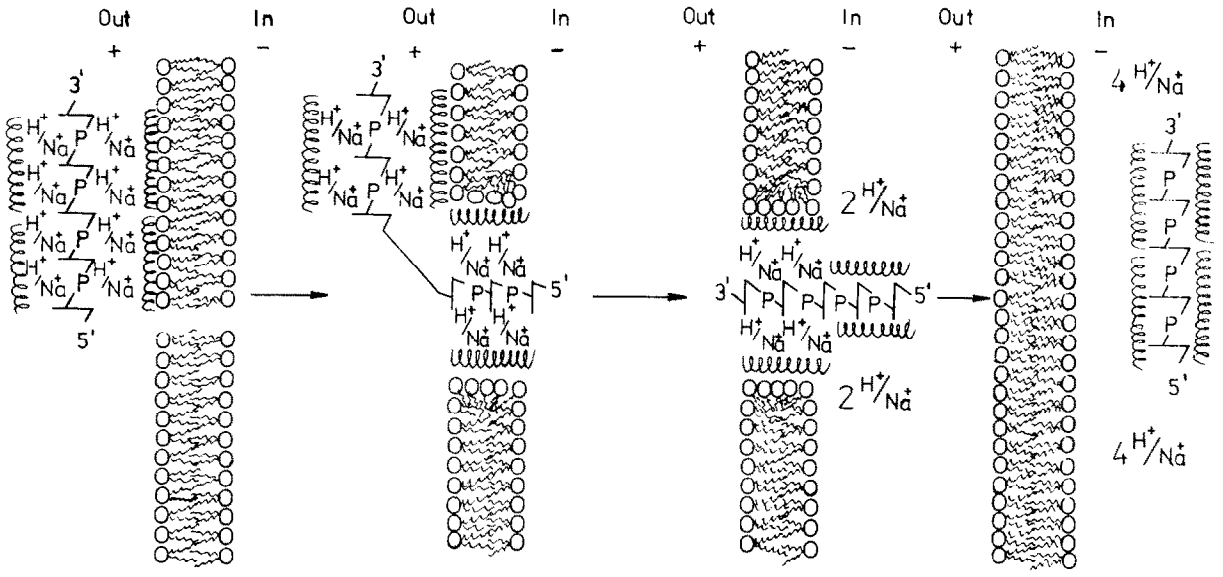

Fig.1. Model of H^+/Na^+ gradient-driven nucleic acid transport. Single-stranded polynucleotide (DNA or RNA) binds H^+ or Na^+ and basic polypeptides at the outer surface of the membrane. Due to interaction with the phospholipid bilayer the nucleoproteidic complex is inserted into the membrane. This effect is probably achieved by altering the phospholipid bilayer structure. Owing to the H^+ or Na^+ electrochemical gradient the positively charged complex moves toward the inner surface of the membrane. The break in the phospholipid bilayer is sealed at the end of nucleic acid transport. Two H^+ or Na^+ are transferred across the membrane together with each phosphate group of the polynucleotide chain.

complex with basic polypeptides by binding H^+ or Na^+ on the outer surface of the cell membrane

(2) Interaction of the polynucleotide–polypeptide complex with the membrane occurs at a site where phospholipid bilayer structure is temporarily interrupted by the transmembrane electric field. The interaction results in the rearrangement of the phospholipid bilayer and in the formation of a transmembraneous channel. This channel exists during the interaction of the polynucleotide–polypeptide complex with phospholipids;

(3) Owing to the difference of the electrochemical potentials across the cytoplasmic membrane, the positively charged polynucleotide–polypeptide complex enters the channel linearly and moves towards the inner surface of the membrane. There the polynucleotide–polypeptide complex liberates either H^+ or Na^+.

In the terms of the above hypothesis, the nucleic acid uptake coupled with H^+ or Na^+ influx is driven by both the membrane potential and transmembrane concentration gradient of either H^+ or Na^+. On the other hand, it seems reasonable that the appearance of electric field-induced local interruptions of the bilayer, which are essential for the interaction of nucleoproteidic complex with the membrane, depends on the membrane potential value. Therefore, at some critical value of the membrane potential a drastic inhibition of nucleic acid transport shoud be observed.

The sensitivity of DNA uptake by bacterial cells to both protonophorous uncouplers of oxidative phosphorylation and the ionophorous antibiotic nigericin [20] supports the proposed hypothesis. According to the mechanism in fig.1, the inhibition of nucleic acid uptake by the uncouplers of oxidative phosphorylation and ionophorous antibiotics occur because of the dissipation of both the membrane potential and the pH gradient.

Interactions between nucleic acid and basic proteins such as histones, nonhistone proteins [66] and the *lac* repressor [67] play an important role in cell chemistry. The accumulated experimental evidence [59,62] indicates the presence of polynucleotide binding proteins in the membranes of cells able to

perform polynucleotide transport. The fact that *H. influenzae* cells [68] but not *Bacillus* and *Diplococcus* species, possess a DNA transport system which is specific for the sequence of purine and pyrimidine bases suggests that cell membrane proteins, binding double-stranded DNA are sequence specific. The most widely accepted DNA–basic protein interaction models at present are those in which the protein exists in an extended chain which wraps itself around the double-helical DNA, covering the narrow groove along the DNA double helix [69,70], or sometimes crossing over to a neighbouring DNA double helix [71], with the positively charged basic side chains of the protein neutralising negatively charged phosphates of the polynucleotide chain. A model of α-helix–double helix interaction, based on the structure of a protamine-transfer RNA complex, has been proposed [72]. In this model the protamine is assumed to be composed of four α-helical segments joined by three partially flexible joints. Each α-helical domain contains four or more consecutive arginine residues, and can fit, approximately, into either groove and can neutralize and hydrogen bond to two negatively charged phosphates across a groove of one double helix. At the same time, the remaining arginines in the same α-helical domain can hydrogen bond and neutralize the negatively charged phosphates of neighbouring double helices.

According to the above concept a similar type of DNA–membrane protein interaction occurs during DNA transport. Some positively charged side chains of the membrane protein or polypeptide are bound to negatively charged phosphates of DNA, while the other side chains can hydrogen bond and neutralize the negatively charged phosphates of the membrane phospholipids.

DNA binding proteins all contain histidine residues which have pK values close to the physiological pH. Therefore histidine seems to be the most likely acceptor of H^+ in the hypothetical polypeptide–polynucleotide complex. Phosphate groups of polynucleotide seem to be Na^+ binding sites in the complex.

The origin of polynucleotide binding proteins, carrying polynucleotide across the membrane, seems to be different. In genetically transformable bacterial species the polynucleotide binding proteins are on the outer surface of the membrane [59,62]. These proteins are assumed to interact with the phospholipid bilayer due to electrostatic interactions and hydrogen bonds. The model is supported by the fact [73–75] that osmotic shocking of bacterial cells, able to take up DNA, releases water-soluble polypeptides of mol. wt $\sim 10^4$ [73]. When isolated, these polypeptides exhibit DNA uptake-stimulating activity after being added to suspensions of bacterial cells devoid of ability to take up DNA. One fraction of DNA uptake-stimulating polypeptides of *B. subtilis* exhibits lytic activity towards isolated cell walls and nuclease activity towards the transforming DNA [75]. Therefore it seems reasonable that some of the nucleic acid binding proteins can exhibit enzymic activity.

On the other hand, it is well known that protoplasts of bacterial and plant cells as well as animal cells take up nucleic acids efficiently only if exogenous basic proteins or polypeptides are added to the incubation medium [76–78]. It seems incredible that stimulation of the nucleic acid uptake by exogenous basic polypeptides occurs due to their activatory effect on an unknown enzyme, a translocase of nucleic acid. In terms of the above concept, basic polypeptides, for example, protamine and polyornithine form a positively charged nucleoproteidic complex during their interaction with nucleic acid and, thereafter, the complex is inserted into the membrane due to the basic polypeptide-induced interruption of the phospholipid bilayer structure. The membrane potential seems to be the driving force that transports the complex because of the inability of basic polypeptides to release H^+ at physiological pH. Following this line of reasoning it seems possible that this primitive mechanism of nucleic acid transport was developed during the early stages of evolution of life and was used by ancient cells to gain and exchange genetic information.

The above concept has been designed in order to describe the transport of 'naked' molecules of the nucleic acid and the nucleoproteidic particles of small viruses. The principles developed may also explain early stages of cell infection by phages possessing contractile tails. Such speculation rests on the experimental evidence [25] indicating the infection of *E. coli* by phage T4 being dependent on the presence of the transmembrane electrochemical gradient of H^+ ($\Delta\bar{\mu}_{H^+}$). Therefore it seems reasonable, that even phage with a contractile tail can use $\Delta\bar{\mu}_{H^+}$ of the recipient cell to introduce its DNA into the cell. In this respect the inner membrane of cells is not a passive wall to be penetrated by phage tails, but plays an active role in phage DNA transport. The task of the

contractile phage tail seems to be to carry the tip of the phage tail across the outer cell membrane to the inner membrane surface to join the tail tube to the membrane. Here the nucleoproteidic complex including the terminus of phage DNA and polypeptide(s) of the phage coat and/or the intrinsic polypeptide(s) of the cell membrane is formed. It seems reasonable that the interaction of the nucleoproteidic complex with the membrane phospholipids leads to transmembrane pore formation. According to the above concept, $\Delta\tilde{\mu}_{H^+}$ is the driving force of phage DNA terminus transport across the membrane.

What component of $\Delta\tilde{\mu}_{H^+}$ will drive the transport of the phage DNA terminus? The answer will depend on the ability of the proteins forming the complex with the phage DNA terminus to bind and release H^+. The transport will depend solely on $\Delta\psi$ if these proteins are only able to bind H^+ but not to release them at physiological pH. If the proteins contain amino acid residues with pK values close to the physiological pH, the dependence on both $\Delta\psi$ and ΔpH will be observed. It is noteworthy that the above concept does not reject the possibility of the involvement of other forces, besides $\Delta\tilde{\mu}_{H^+}$, in cell infection by phages possessing a sophisticated structure. But the uptake of simple bacterial viruses, for example filamentous phages is regarded, according to the above concept, as strictly dependent on the presence of $\Delta\tilde{\mu}_{H^+}$.

The experimental data in [78] indicate that the conjugational DNA transport depends on the intracellular phosphorylation potential as well as on $\Delta\tilde{\mu}_{H^+}$. One can speculate that $\Delta\bar{\mu}_{H^+}$ is needed to drive DNA transport while the phosphorylation potential is involved in the continuous synthesis of DNA which accompanies chromosome transfer. The role of both $\Delta\bar{\mu}_{H^+}$ and the phosphorylation potential in bacterial conjugation is still the object of conjecture.

3.1. *Ca^{2+} gradient-driven transport*

In principle, according to the above concept, nucleic acid transport could be performed by a gradient of any ion, transported down its transmembrane electrochemical gradient. It seem reasonable that involvement of the peculiar ion in transport depends on both the thermodynamic and kinetic factors of the process. The appropriate value of the ion gradient as well as the structure and chemical composition of the cell envelope seem to be most essential.

Several facts concerning key features of Ca^{2+}-induced DNA uptake have been established [5,80–82]:

(1) The presence of Ca^{2+} at 0°C is required for DNA uptake. Ca^{2+} seem to induce specific uptake of DNA rather than cause a nonspecific or irreversible increase in cellular permeability;
(2) The high-temperature step of Ca^{2+}-induced DNA uptake is not strictly dependent on Ca^{2+};
(3) Cells lose Ca^{2+}-induced ability to take up DNA during incubation at 37°C.

It is well known that due to the presence of Ca^{2+}/H^+ antiporter intracellular Ca^{2+} is ~25 μM (reviewed [83]). To induce DNA uptake the cells have to be kept in an ice-cold 50 mM solution of $CaCl_2$ [5]. Here the ratio of extracellular to intracellular Ca^{2+} concentration reaches 2000:1. DNA molecules in the solution strongly bind Ca^{2+}. The free energy change accompanying this process is ~8 kcal/mol [84]. At the same time, the structure of the phospholipid bilayer is greatly influenced by Ca^{2+} concentration in the surrounding medium. For example, there is evidence [85] that the effect of Ca^{2+} on the membrane is related to its ability to induce phase transitions and a phase change from fluid to crystalline acyl chain packing in negatively charged phospholipid layers. It has been proposed [85,86] that this effect induces a transient destabilization of the bilayer. The molecular events responsible for creation of this unstable state have been regarded [85] as three related phenomena:

(1) Ca^{2+} concentration gradient across the phospholipid bilayer;
(2) The boundaries between two solid and fluid phospholipid domains;
(3) The transient local release of heat, liberated by the exothermic crystallization of the phospholipid acyl chains.

The experimental evidence in [87] indicates that Mg^{2+} and presumably Ca^{2+} up to 80 mM stimulate polynucleotide binding to the phospholipid vesicles while Na^+ causes significant inhibition of the binding. Despite the absence of direct proof the data in [87] make it highly probable that K^+ can also prevent the binding of DNA–divalent cation complex to the membrane of the phospholipid vesicle.

Consideration of the above experimental observations leads to the hypothetical mechanism of Ca^{2+} gradient-driven DNA transport (fig.2) which rests on the principles of nucleic acid transport formulated above. According to this concept, there is no DNA translocase in the membrane. The key point of the proposed mechanism is the interaction of the electroneutral DNA–Ca^{2+} complex with the membrane

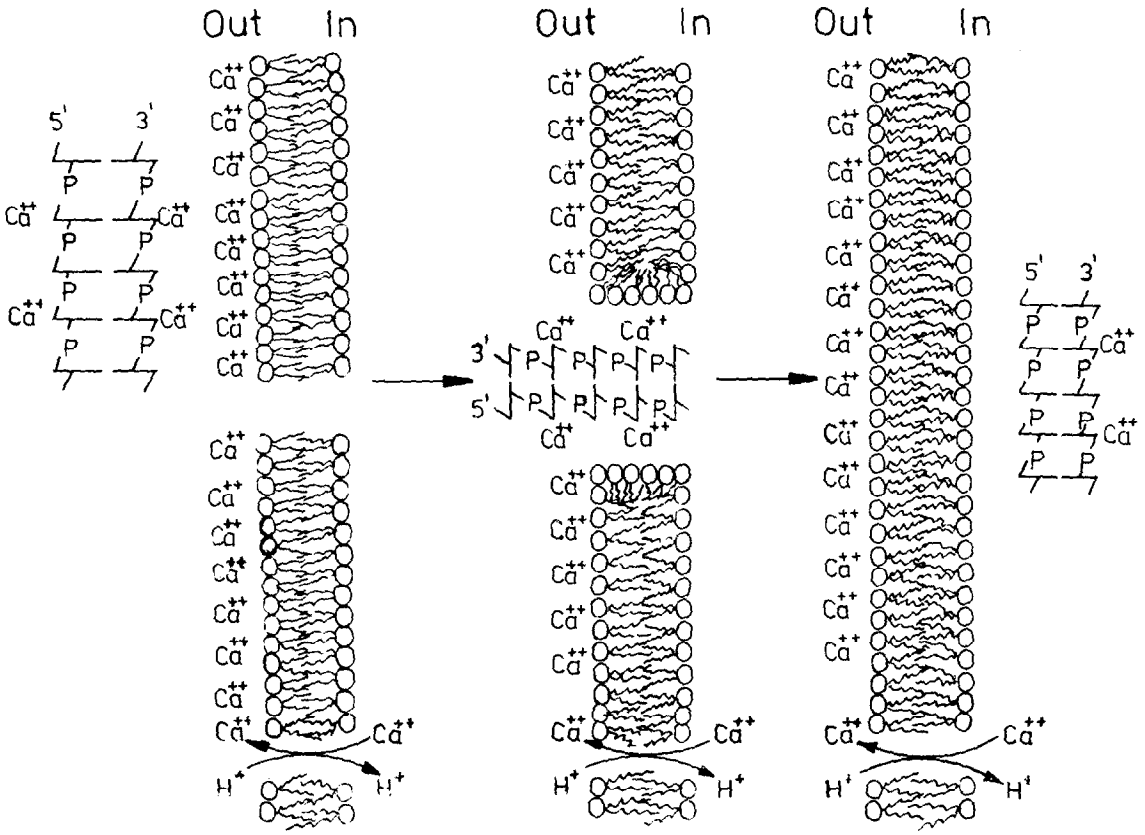

Fig.2. Model of Ca^{2+} gradient-driven DNA transport. An excess of Ca^{2+} is present outside the cell. Ca^{2+} binds to the DNA and the membrane phospholipids and forms electroneutral complexes. One Ca^{2+} is bound per two phosphate groups of the DNA molecule. The complex interacts with the membrane phospholipids and alters the bilayer structure. During insertion of the complex into the membrane bonding occurs between Ca^{2+} and phosphate groups of the DNA and the phospholipid molecule. One Ca^{2+} is transferred across the membrane together with two phosphate groups of the DNA.

at a site where the bilayer structure is interrupted by Ca^{2+}. Due to the interaction of the complex with phospholipids a rearrangement of phospholipid molecules occurs. The process proceeds more easily at 0°C while the interaction between hydrocarbon chains of phospholipids weakens. The presence of Ca^{2+} is obligatory during this step. The interaction of the DNA–Ca^{2+} complex with phospholipids results in the temporary formation of a transmembranous channel letting the DNA–Ca^{2+} complex enter the cytoplasm while binding of the complex at the inner surface of the phospholipid bilayer is prevented because of the high intracellular K^+ concentration (fig.2). Inside the cell the dissociation of DNA–Ca^{2+} complex seems to be promoted by two factors:

(1) Intracellular K^+ concentration;

(2) Efflux of free Ca^{2+} due to the Ca^{2+}/H^+ exchange.

Therefore, owing to the electroneutrality of DNA–Ca^{2+} complex, there is no reason to expect an influence of $\Delta\psi$ on transport. According to the above mechanism, the transmembrane concentration gradient of Ca^{2+} is the driving force of the transport under such conditions. ΔpH plays an essential role, preventing the equilibration of Ca^{2+} concentrations on both sides of the membrane.

Therefore, a slight inhibitory effect of the uncouplers on Ca^{2+}-induced DNA uptake [58,80] seems to be related with the dissipation of transmembrane Ca^{2+} gradient.

To induce DNA transport both the presence of high Ca^{2+} concentrations and drastic changes of temperature are needed. Therefore the biological signif-

icance of Ca^{2+}-induced DNA uptake seems to be very limited. On the other hand, many of the recent techniques for the in vitro manipulation of genetic material rely ultimately upon the uptake by Ca^{2+}-treated cells of modified DNA.

4. Conclusion and research prospect

The above hypothesis postulates the transmembrane electrochemical gradient of ions to be the driving force of nucleic acid transport. The ion which drives nucleic acid transport seems to be H^+ in bacteria, Na^+ in animal cells and Ca^{2+} under conditions of artificially imposed transmembrane Ca^{2+} gradient. The hypothesis postulates that the nucleic acid-permeable channel does not pre-exist in the membrane. It is formed during the interaction between the nucleic acid and the membrane constituents.

Several ways to verify the hypothesis can be proposed:

(1) The direction of nucleic acid transport will change according to the change of the direction of transmembrane electrochemical gradient of the ion which drives the transport. For example, DNA taken up by the bacteria in the presence of $\Delta\psi$ (plus outside) and ΔpH (acid outside) will be extruded from the cell if $\Delta\psi$ (plus inside) and ΔpH (acid inside) are imposed;

(2) The uptake of nucleic acid by bacterial or animal cells coupled with the influx of H^+ or Na^+, correspondingly, will depend on values of $\Delta\tilde{\mu}_{H^+}$ or $\Delta\tilde{\mu}_{Na^+}$, but not on the ratio of the values of $\Delta\psi$ and ΔpH (ΔpNa);

(3) Nucleic acid uptake will not depend on the value of intracellular phosphorylation potential.

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