Biochimica et Biophysica Acta 467 (1977) 251—261

BBA 77707

PROTON-INDUCED UPTAKE OF MAMMALIAN DNA BY DOG ERYTHROCYTE PINK GHOSTS

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(Received November 18th, 1976)

Summary

Low pH (below 6) induces the uptake of mammalian DNA in dog erythrocyte pink ghosts. Uptake requires either Ca^{2+} or Mg^{2+} and is stimulated by ATP. These agents induce a rapid sphering of the ghosts at 37°C and sphering is required for uptake. Uptake is increased in ghosts which have been incubated 60—90 min before adding the DNA. Uptake is strongly temperature-dependent. Lowering the temperature of a suspension of ghosts taking up DNA at 37—0°C stops uptake. It is concluded that uptake depends on active membrane processes and that it may depend on the capacity of the ghosts to maintain cation exchange.

Introduction

Understanding the mechanism by which DNA is taken up by cells is important in problems related to the control of virus infection and to transfer of genetic information. Genetic studies with bacterial transforming system have shown that the uptake is determined both by base composition [1] and by the degree of strandedness [1,2]. It has also been found that in both bacterial and mammalian cells, DNA uptake is stimulated by Ca^{2+} [3,4] by certain organic polycations [5,6] and by some drugs [7]. These agents have been assumed to act either by reducing the negative charge on the DNA and thus facilitating its passage through the membrane or by forming "holes" in the membrane through which it can diffuse into the cells. These mechanisms would not account for the specificity which uptake systems exhibit with respect to the physical characteristics of the DNA.

It is difficult in cellular systems to investigate either the action of these agents or the mechanisms involved in uptake. The way in which DNA interacts with the membrane, any change it might induce and the energetics involved in

the actual translocation through the membrane cannot be investigated in cells where so many other processes are occurring.

We report here that low pH (below 6) induces uptake of double-stranded mammalian DNA by dog erythrocyte ghosts suspended in hypotonic saline in the presence of ATP and a divalent cation. The finding may be related to the finding of Schell that low pH induces the uptake of synthetic polyribonucleotides in Ehrlich Ascites cells [8]. The proton-induced uptake in these ghosts may provide a useful system for studying aspects of the membrane-DNA interaction important in uptake in cellular systems.

Materials and Methods

Preparation of pink ghosts

Erythrocyte ghosts were prepared from heparinized blood from two dogs. Each dog was bled (20 ml) not more than three times in 1 week and then rested for at least 10 days. They were given weekly injections of an iron dextran preparation ("Nonemic"). The blood was stored at 8° C and used within 3 days. The red blood cells were washed and diluted 4 times in cold isotonic phosphate according to Schrier [9]. The ghosts were prepared by a modification of Schrier's step-wise hemolysis procedure using the following solutions: (1) 0.05 M NaCl, pH 6.9; (2) 0.02 M NaCl with 0.5 mg/ml bovine serum albumin; (3) 0.01 M NaCl with 0.25 mg/ml bovine serum albumin, pH 6.8–6.9. The exact procedure, working with solutions and cells kept on ice was as follows: 10 ml of solution 1 was added rapidly to 0.5 ml of the washed, diluted blood cells. After 7 min, the solution was diluted twice with solution 2 and after 7 min again diluted twice with solution 3. After 7 min it was centrifuged at 12 000 × *g* for 7 min. The supernatant fluid was aspirated from the pink pellets which were resuspended in 20 ml solution 3. The suspension was held for 30 min on ice and sedimented. The pellets were combined and washed in 10 ml solution 3. In experiments where more than one solution was to be tested, the suspension in solution 3 was divided before the final centrifugation.

Preparations of [^{3}H]- or [^{14}C]DNA from cultures of WI-L2 lymphocytes, grown in RPMI medium supplemented with 8% fetal calf serum were made by several different methods. The cells were labeled during log phase when they had reached a density of 6–8 · 10^5/ml by adding either 0.7 μCi/ml of [*Me*-^{3}H]-thymidine, or 0.1 μCi of [*Me*-^{3}H]thymidine. Incubation was continued for 18 h during which the population doubled. The cells were harvested by centrifuging for 5 min at 120 × *g* and washed in balanced salt solution.

Preparation A was made according to the Marmur procedure [10]. Alkaline sucrose gradient analysis using purified adeno-2 virus DNA (kindly provided by Dr. L.D. Hodge) as a marker showed that it had the usual broad low peak with about 80% of the material between 6 and 12 million daltons. The specific activity was 7.6 · 10^4 cpm/μg and the absorbance 260/280 was 1.90.

Preparations B, D and J were made from nuclei isolated from washed cells resuspended at 1 · 10^6 cells/ml in cold 0.1 M NaCl/0.05 M EDTA, pH 6.5, by lysing with 0.3% Nonidet P-40. The nuclei were sedimented, resuspended at 10-times the original volume in 0.15 M NaCl/0.15 M EDTA, pH 8.5, and digested with proteinase K according to the procedure of Gross-Bellard [11], and depro-

teinization according to Marmur. The material was very viscous and had an absorbance 260/280 of 1.94 (B); 1.92 (D) and 1.96 (J). The contaminating protein was not decreased by further treatment with proteinase K or pronase. Alkaline sucrose gradient analysis showed peaks at 10—15, and 20—25 million (B and D) and with (J) also at 40—60 million daltons. Specific activity of [^{3}H]-DNA (B) was $2.4 \cdot 10^4$ cpm/μg, of [^{3}H]DNA (D) was $1.2 \cdot 10^4$ cpm/μg and of [^{14}C]DNA (J) $1.0 \cdot 10^4$ cpm/μg.

DNA preparations were stored in 70% ethanol at −20° C. They were solubilized in sterile 0.03 M NaCl/0.01 M sodium phosphate buffer, pH 7.4. The pH was adjusted on aliquots by slow addition of 0.1 M HCl at the beginning of each experiments.

Radiolabeled DNA was assayed by scintillation counting of samples (0.1—0.15 ml) dissolved in Aquasol (New England Nuclear).

Assay of DNA uptake determined as radiolabeled DNA lost from the supernatant

The ghost suspensions placed in Beckman centrifuge tubes were incubated in a constant temperature water bath. The radiolabeled DNA was added and mixed by blowing gently through the delivery pipette. The ghosts remained uniformly dispersed, but at the time of sampling the suspensions were mixed by blowing very gently through a pasteur pipette.

The amount of radioactive DNA added to the suspension was determined by assay of two 0.1 ml samples taken at t_o. At various times during the incubation, 0.2 ml samples were removed using an automatic pipette. The samples were centrifuged for 7 min at 12 000 $\times g$. The DNA remaining in the supernatant was assayed on 0.1 ml samples, and the DNA taken up calculated by subtraction from the amount added.

Radiolabeled-DNA recovery in ghosts

The supernatant fluid was aspirated from the pelleted ghosts, which were then resuspended using a vortex mixer in 0.5 ml of 2% sodium dodecyl sulfate, and 0.15 ml was taken for assay. It was not found necessary to wash the pelleted ghosts before assaying as the error due to trapped fluid or drainage from the tube was negligible. This was demonstrated by the finding that assays of ghosts pelleted from suspensions which had not taken up any DNA showed repeatedly that the counts trapped in the ghosts were less than 1% of the total counts added.

Special chemicals

Proteinase K was obtained from E.M. Laboratories. DNAase was the RNAase-free Wortington product. Radiolabeled thymidine was obtained from New England Nuclear. Nonidet P-40 (NP-40) was obtained from the Shell Chemical Co. All other reagents were obtained from Calbiochem. "Nonemic" was obtained from J.A. Webster Co.

Results

Effect of pH dn the uptake of [^{3}H]DNA

Dog erythrocyte ghosts suspended in neutral salt solutions do not take up

DNA even after several hours of incubation at 37°C. Lowering the pH of the suspension was found to induce uptake. However, the amount taken up varied between suspensions and with a given preparation of ghosts as a result of what seemed to be minor changes in the experimental procedures. Results appeared to be more consistent when the ghosts were suspended in hypotonic saline or phosphate in the presence of divalent cation and ATP. As a result of these preliminary studies, solution A was adopted for further studies. This contained 30 mM NaCl, 2 mM $MgCl_2$, 5 mM ATP, and 0.8 mM sodium phosphate buffer, formulated according to the Henderson-Hasselbach equation to give the desired pH. Final adjustment of pH was done using 0.1 M NaOH or HCl. The preparation of the ghost suspension was also standardized. The pelleted membranes were kept on ice and used within 15 minutes of the last step of the hypotonic lysis procedure. They were suspended directly into the solution to be tested, and were incubated for 15 min at 37°C before the radiolabeled DNA was added.

The inducing effect of protons on the uptake of [^{3}H]DNA is shown in the data presented in Fig. 1, where uptake by ghosts suspended at four different pH values is followed with time. It is seen at pH 6.2 uptake is slow, and that not more than 20% of the [^{3}H]DNA is taken up during the first 90 min of incubation. Decreasing the pH of the suspension increases the rate of uptake. At pH 4.8, more than 80% of the [^{3}H]DNA added is taken up during the first 10 min, and 98% is taken up after 45 min.

The uptake of DNA induced at low pH is temperature dependent, as is shown in Fig. 2, where uptake by a suspension of ghosts (pH 5.4) at 37°C and

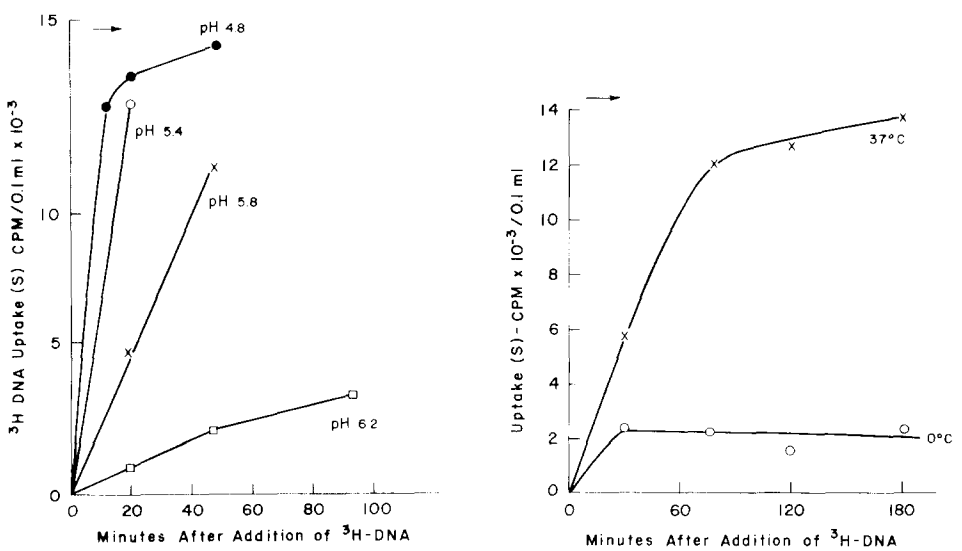

Fig. 1. Proton induced uptake of [^{3}H]DNA by dog erythrocyte pink ghosts. Equal volumes of pelleted ghosts membranes suspended in solution A at pH 6.2 (□); pH 5.8 (-X-); pH 5.4 (○); and pH 4.8 (●); incubated 15 min at 37°C and supplemented with 3 μg/ml [^{3}H]DNA (preparation A). Uptake determined as loss of [^{3}H]DNA from solution (S). Arrow indicates cpm/0.1 ml added.

Fig. 2. Effect of temperature on proton induced uptake of [^{3}H]DNA by dog erythrocyte ghosts. Ghost membranes suspended in solution A, pH 5.4, at 37°C (-X-) and at 0°C (-○-). Incubated for 15 min before addition of [^{3}H]DNA (see legend of Fig. 1).

at 0° C is compared. At 37° C, uptake proceeds until virtually all of the added [^{3}H]DNA is removed from solution. In contrast, ghosts kept at 0° C taken up [^{3}H]DNA slowly during the first 20—30 min and then stop.

Using phase contrast microscopy, it was seen that when ghosts are suspended in solution A at pH values below 6, and kept at 37° C, they convert from the collapsed membranes produced by hypotonic lysis to smaller and uniform spheres. Quantitative studies were not done, but the rate of sphering and the percentage of the population converted appeared to increase directly with decreasing pH. At pH 6.2, only a few ghosts sphered even after several hours of incubation, wheras at pH 5.4, all of the ghosts sphered after 5—10 min incubation at 37° C. This correlation between the effects of protons on the sphering of ghosts and on DNA uptake suggested that uptake might depend upon the sphering process. Inspection of the ghosts kept at 0° C, where the uptake of the ghosts was greatly reduced, showed this to be the case; most of the membranes remained collapsed and none of the ghosts sphered completely.

Requirement for Ca^{2+} or Mg^{2+} in uptake

The data presented in Fig. 3 show that uptake of [^{3}H]DNA at low pH requires either Ca^{2+} or Mg^{2+}. After the addition of the [^{3}H]DNA, ghosts suspended in solution A without Mg^{2+} showed a lag of 30—40 min before uptake started. Addition of either 1 mM $CaCl_2$ or 1 mM $MgCl_2$ abolished the lag, and

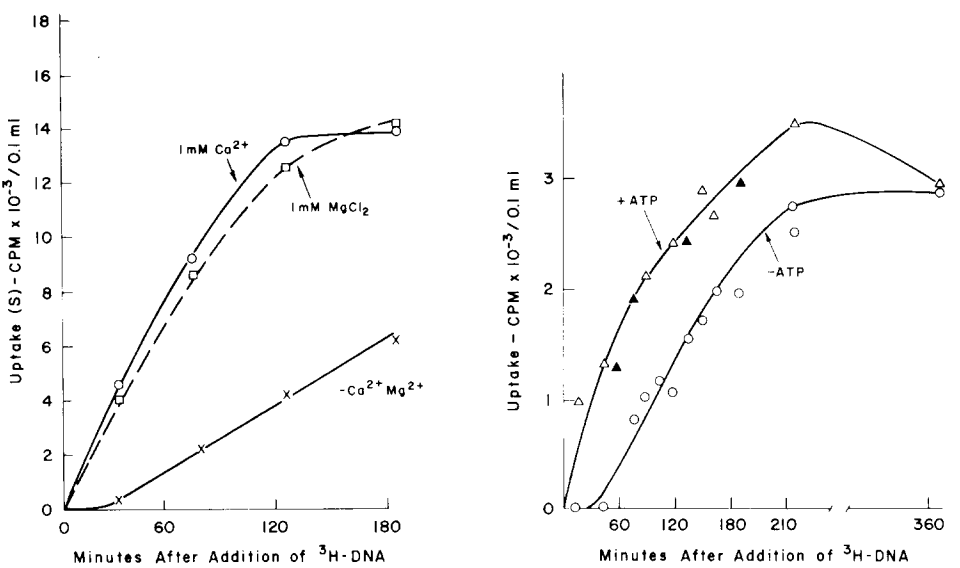

Fig. 3. Requirement for Ca^{2+} or Mg^{2+} for proton-induced uptake of [^{3}H]DNA. Equal volumes of ghost membranes suspended in solution A (pH 5.4) made without divalent cation (-X-); or with 1 mM of $CaCl_2$ (-○-) or 1 mM $MgCl_2$ (-□-).

Fig. 4. Stimulating effect of ATP on uptake of [^{3}H]DNA by dog erythrocyte ghosts at pH 5.4. Uptake of [^{3}H]DNA by ghosts suspended in solution A with (△ ▲) or without 5 mM ATP (○). Ghosts incubated 15 min at 37°C before addition of [^{3}H]DNA (Preparation 2) at 20 μg/ml. Solid symbol (▲) indicates [^{3}H]-DNA in ghosts and open symbols (△ ○) indicate amount of [^{3}H]DNA taken up from solution (see Materials and Methods).

uptake began as soon as the [^{3}H]DNA was added, as expected from the previous experiments.

Inspection of the ghosts showed that a divalent cation is required for sphering. Suspended in the absence of either Ca^{2+} or Mg^{2+}, the ghosts sphered very slowly and the process was not complete even after several hours of incubation. Addition of either Ca^{2+} or Mg^{2+} stimulated sphering and at pH 5.4 with 1 mM of either Ca^{2+} or Mg^{2+} all the ghosts had sphered within the first 15 min.

Stimulation of [^{3}H]DNA uptake by ATP

The effect of ATP on the uptake of DNA is shown in Fig. 4. The ghosts were suspended in solution A, pH 5.4, made with and without 5 mM ATP. It is seen that in the presence of ATP, uptake started as soon as [^{3}H]DNA was added, whereas in the paired suspension without ATP there was a lag of more than 45 min before uptake started. However, after uptake started it proceeded at essentially the same rate as in the presence of ATP.

These kinetics suggest that uptake may involve two processes; an early phase which requires added ATP and a later phase which does not. Several considerations suggest that the early ATP-dependent phase could be related to the sphering of the ghosts. In a number of ghost systems, addition of ATP and either Ca^{2+} or Mg^{2+} has been found to cause rapid changes in the conformation of membrane proteins [12], induce a reorganization of the internal membrane disorganized by hypotonic lysis [13], and cause contraction of the ghosts [14,15]. Furthermore, since the binding sites for ATP are on the inside membrane [16,17] and resealed ghosts are impermeable to ATP [17—19], any effect of ATP would be limited to the early phase of recovery before the membranes have resealed. Inspection of the ghosts showed that ATP did stimulate sphering. In the absence of ATP, ghosts took nearly 30 min instead of the 10—15 min to sphere as compared to 10—15 min observed in the presence of ATP. However, the delay in sphering did not account completely for the difference in kinetics since sphering appeared to be complete after 30 min, but uptake did not start until after 45 min. This suggests that uptake depends on some process in addition to sphering, which takes time to become established in the ghosts following hypotonic lysis.

Uptake in the second phase, when he ghosts have already sphered, is strongly temperature dependent. Lowering the temperature of the suspension from 37 to 0°C stops uptake (Fig. 5).

Effect of preincubation of ghosts on uptake of [^{3}H]DNA

In the previous experiments the ghosts were preincubated at 37°C for 15 min before the addition of the DNA. Observation of many preparations of ghosts, suspended in solution A at pH values below 5.4, showed that the sphering was completed within this time. Complete resealing of the membrane following hypotonic lysis, however, would be expected to take longer, as indicated by studies with human erythrocyte ghosts [17—20]. The finding that uptake depended not only on the sphering of the ghosts but on a second membrane process that developed more slowly during incubation of the ghosts, suggested that uptake might depend on the resealing of the membrane. If this were the case, ghosts which had already resealed should take up DNA better than

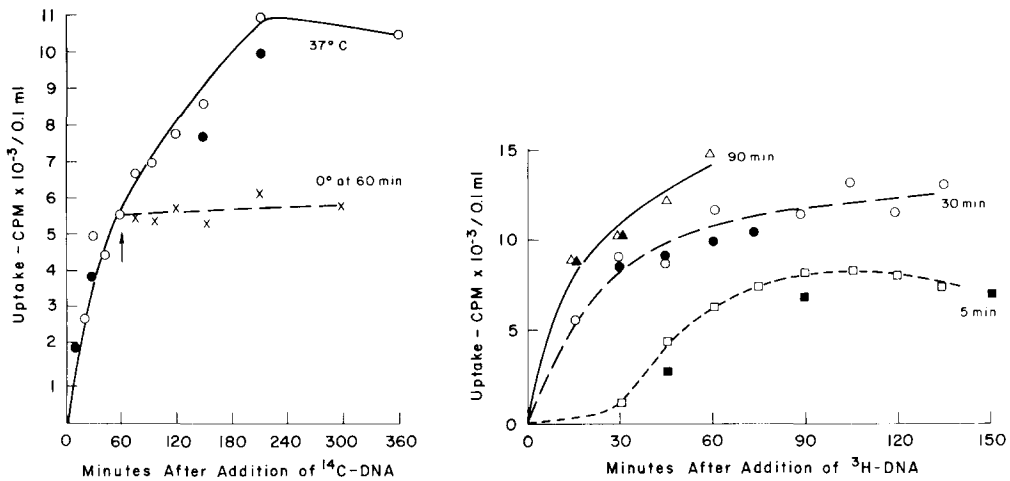

Fig. 5. Temperature dependence of late phase of uptake of [^{14}C]DNA by dog erythrocyte ghosts. Ghosts suspended in Ca^{2+} supplemented solution A, pH 5.3, and incubated 15 min at 37°C before addition of [^{14}C]DNA (J) at 15 μg/ml. At 60 min a portion was placed at 0°C. Uptake in suspension at 37°C determined as [^{14}C]DNA lost from solution (○) and by assay of ghosts (●). In suspension at 0°C, values for [^{14}C]DNA in ghosts (X).

Fig. 6. Effect of prolonged pre-incubation of ghosts on uptake of [^{3}H]DNA. Ghosts suspended in a Ca^{2+}-supplemented solution A at pH 5.3 and incubated at 37°C. Aliquots removed and supplemented with 8 μg [^{3}H]DNA (solution B) after 5 min (□ ■), 30 min (○ ●) and 90 min (△ ▲). Solid symbols equal [^{3}H]-DNA in ghosts. Open symbols equal [^{3}H]DNA in ghosts.

ghosts which had only just sphered. This possibility was investigated by determining the uptake of [^{3}H]DNA in suspensions of ghosts which had been allowed to incubate at 37°C for more prolonged periods before the addition of the [^{3}H]DNA.

The results are shown in Fig. 6. In this experiment the ghosts were suspended in solution A pH 5.4, which had been supplemented with 0.2 mM $CaCl_2$. The Ca^{2+} was added in view of the evidence that low concentrations of Ca^{2+} in addition to Mg^{2+} may be required for resealing of ghost membranes [18,20]. The suspension was divided into three portions which were incubated at 37°C for 5, 30, and 90 min before the [^{3}H]DNA was added. It is seen that the rate and amount of [^{3}H]DNA taken up increased with the time the ghosts were allowed to incubate before the [^{3}H]DNA was added. Preincubation for at least 60 min was needed before the ghosts had gained the maximum capacity to take up [^{3}H]DNA.

An experiment was done (Fig. 7) to determine whether the increased capacity of the preincubated ghosts to take up DNA depended on temperature-dependent changes occurring in the membrane which would be associated with membrane recovery or whether it was due to some passive physical change. A suspension of ghosts in the Ca^{2+}-supplemented solution A, pH 5.3, was divided into three portions (a,b,c), Two were placed at 37°C and their capacity to take up [^{3}H]DNA tested after 5 min of incubation (a) and after 95 min (b). c was kept at 0°C for 95 min. As shown in the previous experiment, ghosts which had been preincubated for 95 min at 37°C showed a much greater rate of uptake than the ghosts which had been preincubated only 5 min. However, the ghosts

which had been kept in the cold for 95 min shifted to 37° C and tested after 5 min of incubation were found to take up [^{3}H]DNA very slowly.

Inspection of the ghosts held at 0° C showed that they underwent a very slow contraction and 5 min after they were placed at 37° C the sphering appeared to be complete. The failure of these ghosts to take up DNA indicates that the enhanced capacity exhibited by ghosts after incubation at 37° C depends on temperature-dependent changes in the membrane related to some process occurring after sphering. Resealing of human erythrocyte has been found to depend on incubation at 37° C and not to take place in the cold. This may suggest that the capacity of these dog erythrocyte ghosts to take up DNA depends on the degree to which the membranes have resealed.

Stability of ghost-bound DNA

The stability of the DNA associated with the ghosts during the proton-induced uptake process has been tested by determining whether it was released by added DNAase. In these experiments ghosts were suspended in the Ca^{2+}-supplemented solution A, pH 5.0, containing 8 mM, rather than 2 mM, $MgCl_2$. The Mg^{2+} was increased in view of the evidence [21] that 8 mM Mg^{2+} is needed for maximum activity of the enzyme at pH values below 5.5.

The activity of the enzyme was tested in this solution at pH 5.0. With 20 μg/ml of the DNAase and 8 μg/ml of [^{3}H]DNA, it was found that 65% of the

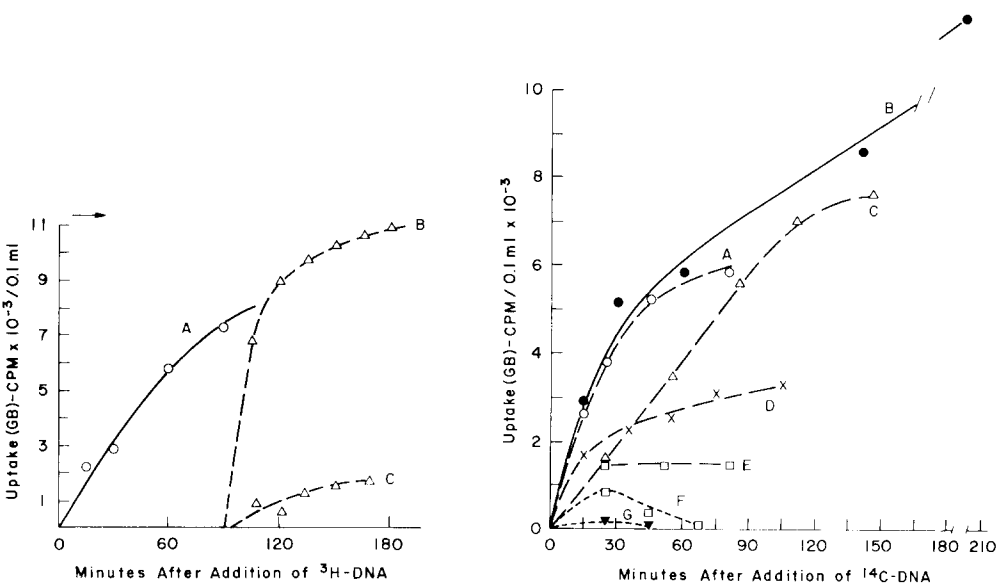

Fig. 7. Effect of temperature during pre-incubation on capacity of ghosts to take up [^{3}H]DNA. A suspension of ghosts in Ca^{2+}-supplemented solution A was divided into 3 portions (a,b,c). Portions a and b were pre-incubated at 37° C for 5 minutes (A) and for 95 min (B) before [^{3}H]DNA (preparation B) was added. Portion c was kept at 0° C for 95 min. It was then shifted to 37° C and incubated for 5 min before addition of [^{3}H]DNA (C). Time given in minutes after addition of [^{3}H]DNA to portion a.

Fig. 8. Inactivation of [^{14}C]DNA during storage. A solution of [^{14}C]DNA (J) at 80 μg/ml was stored at 8° C. Uptake by "standard" suspension of ghosts tested at 8 μg/ml at different times. Uptake given as cpm/0.1 ml of [^{14}C]DNA lost from the supernatant. Assayed at day 0 (curve A); 30 (curve C); 45 (curve D) 55 (curve E); 60 (curve F) and 70 (curve G). Curve B, uptake of 20 μg/ml at day 0.

TABLE I

EFFECT OF DNAase ON [^{3}H]DNA IN GHOSTS

A suspension of ghosts in Ca^{2+}-supplemented solution A (pH 5.0) was given 8 μg/ml [^{3}H]DNA (10 056 cpm/0.1 ml). At 30 min DNAase (20 μg/ml) added to one-half of the suspension and [^{3}H]DNA remaining in ghosts followed with time.

Minutes	Ghost bound cpm/0.1 ml	
	Control	+ DNAase (30 min)
15	7 763	
30	8 912	
45	8 580	8 412
60	6 807	9 066
75	8 190	7 471
90	7 391	7 541
105	7 093	6 849

[^{3}H]DNA was degraded to trichloroacetic acid-soluble material in the first 15 min and 76% was degraded after 45 min (Values are ±5%).

In the experiment presented in Table I, the ghosts were given 8 μg/ml of [^{3}H]DNA. Uptake was completed within 30 min. Of the 10 300 cpm/0.1 ml added, 8912 cpm/0.1 ml, or 85%, were recovered on assay of the ghosts. At this time, a portion of the suspension was taken and supplemented with 20 μg/ml of DNAase. Assay of the control suspension showed that the amount of [^{3}H]DNA recovered in the ghosts decreased by about 10% during the remaining 90 min of incubation. This may be attributed to both an increasing "stickiness" of the ghosts so that some are lost during pipetting, and to the fragility of the ghosts under these conditions. It is seen, however, that the loss was no greater in the suspension incubated with DNAase. If the [^{3}H]DNA had been accessible to the enzyme, more than 76% would have been lost during the first 45 min.

The finding that the DNA associated with the ghosts is not released by DNAase does not prove that the DNA taken up is inside the ghost. It could be buried in the outside membrane in some way so as to be occluded from the enzyme.

Effect of age of radiolabeled DNA on uptake

Considerable variability was encountered in these studies with respect to the total amount of DNA taken up by ghosts in different experiments. This was considered at first to be due to the variability in the "quality" of the ghost preparations. Inspection of the data showed that in all cases where uptake was anomolously low, the solution of DNA used had been frozen or thawed several times, which suggested that uptake might depend on the physical state of the DNA.

This possibility was investigated more systematically by the experiment presented in Fig. 8, where uptake was assayed periodically during the storage of a solution of [^{14}C]DNA. The [^{14}C]DNA had been solubilized in sterile 0.03 M NaCl/0.01 M sodium phosphate buffers, pH 7.4, divided into aliquots and stored at 8°C. A separate aliquot was used for each assay. The DNA was assayed using ghosts suspended in the Ca^{2+}-supplemented solution A, pH 5.3,

which had been preincubated for 15 min before the addition of the [^{14}C]DNA at 8 μg/ml. Assay of the newly solubilized material showed that 85% was taken up after 75 min (curve A) and extrapolation showed that more would have been taken up if the incubation had been continued. After addition of 20 μg/ml (curve B), the ghosts were still taking up the DNA after 3 h. There was no significant change in the uptake during the first 30 days. Tested at 30 days (curve C) the rate was slower but the 8 μg/ml added was eventually taken up. During the second month of storage the percentage of the DNA added that was taken up declined rapidly. Tested at 60 days, only 5% was taken up.

The ghost suspension which showed no uptake of the 60-day-old [^{14}C]DNA was tested with two newly solubilized [^{3}H]DNA preparations; one from WI-L2 and one from Hela Cells. Tested at 20 μg/ml, the ghosts took up 42% of the WI-L2 and 53% of the Hela DNA in the first 45 min. This shows that the failure to take up the old [^{14}C]DNA was not due to the ghosts but to changes occurring in the DNA during storage.

The old [^{14}C]DNA was still viscous and all of the counts were precipitated with cold trichloroacetic acid. The decline in uptake could not be ascribed to gross degradation but would appear to be due primarily to radiation-induced damage. This was further suggested by the finding that with two preparations of [^{3}H]DNA the capacity to be taken up was lost during storage in 70% ethanol at −20°C. Physical measurements were not done to determine the changes involved, but these findings suggest that uptake depends on the integrity of the double-stranded molecules.

Discussion

The uptake of DNA by dog erythrocyte pink ghosts which is induced at low pH is not adequately explained as due to passive processes resulting from surface charge changes. Such changes would be completed fairly rapidly whereas uptake was found to continue for several hours. Uptake was also found to be strongly temperature dependent suggesting that it depends on active membrane processes. This was further suggested by the finding that it depends on two physical changes in the membrane, e.g., sphering and a slower heat-dependent change, which have been shown in studies on human erythrocyte ghosts to be involved in the recovery of the membranes and resumption of the cation pumps [13,17—20]. These findings suggest that uptake of DNA depends in some way on the capacity of the ghost to maintain cation exchange.

This conclusion, however, raises an immediate difficulty. The uptake of the negatively charged DNA indicates the presence of an electro-positive transmembrane field. The exchanges taking place under the conditions used here, however, would be electro-neutral. Thus, uptake of the DNA cannot be coupled to exchanges normally maintained by the ghosts. The continued uptake suggests that the DNA alters the normal membrane processes possibly by inducing changes in membrane conductance.

This possibility was suggested by the effects of the Exciting Inducing Material on artificial membranes [22,23]. This material, later shown to be a ribonucleoprotein, can induce 10^{7}-fold increase in cation conductance and may decrease the permeability of the membrane to anions. If DNA induced similar

conductance changes in the ghost membrane, it would give rise to the positive transmembrane field needed, but uptake would also depend on the ghost being able to maintain some efflux of cations. The rate and extent of uptake would depend on the interplay between these two processes. Given conditions where the ghosts could not compensate for the electrogenic change induced by DNA, the initial electrostatic binding of the DNA would result in binding of more DNA. The interaction could result, however, in the development of a positive transmembrane field intensive enough to pull the molecule completely through the membrane into the ghost. The uptake would be the result of a process induced by the DNA and determined both by the physical characteristics of the DNA and by membrane energetics.

Acknowledgements

This investigation was supported by National Institutes of Health Grant GM 19481 awarded to E.A. Adelberg, J.M. Eisenstadt, D. Hodge, P.T. Magee, and F. Ruddle for coordinated efforts in mammalian gene transfer.

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