Towards Proto-Cells: "Primitive" Lipid Vesicles Encapsulating Giant DNA and Its Histone Complex

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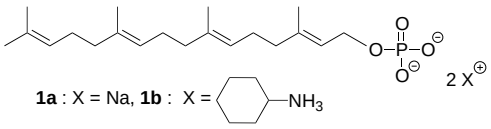
Among the initial stages in the formation of proto-cells on Earth, the encapsulation of DNA molecules from the surrounding environment into closed systems appears to be essential, and several procedures for the encapsulation of DNA into liposomes have been described.[1] For example, Deamer and Barchfeld employed the dehydration/rehydration method to trap salmon sperm DNA (ca. 20 kb) into egg lecithin vesicles,[1a] Luisi's group studied the entrapment of a plasmid DNA (3.3 kb) by using the same method, the reverse-phase method and the freeze/thaw method,[1b] and Jay and Gilbert observed the enhancement of incorporation of DNA (1 kb) in the presence of a basic protein, lysozyme.[1c] DNA – lipid complexes have also been considered to achieve transport of DNA into cells, in particular for gene transfer therapy.[2] However, conventional vesicles with a size of several tens of nanometers are too small to incorporate natural genomic DNA molecules containing 10^5 – 10^8 bp (their full length being on the order of 100 μm – 10 cm). In order to study DNA incorporation into model proto-cells, it is necessary to (1) prepare giant vesicles with a diameter of several micrometers, into which "giant" DNAs can be entrapped smoothly, and (2) monitor in real time individual DNA molecules entrapped within these giant vesicles. Recently, we have found that giant DNAs larger than 100 kb are entrapped spontaneously into giant vesicles of neutral phospholipids in the presence of magnesium ions.[3a] In this last study, we could not control the conformation of the entrapped DNAs. We now report two efficient methods for preparing a primitive cell model entrapping large DNA: natural swelling of polyprenyl phosphates in the presence of DNAs, and laser manipulation. We show that DNA and the DNA – histone complex can be encapsulated into giant vesicles, where they assume "elongated-coil" and "folded-compact" conformations, respectively. In this study, we have used as lipid membrane components the disodium and dicyclohexylammonium salts of geranylgeranyl phosphoric acid (**1 a** and **1 b**, respectively), which we have postulated to be a "primitive" membrane lipid.[4]



We have prepared giant vesicles entrapping bacteriophage T4 DNA (166 kb, contour length 57 μm),[3] by spontaneous swelling of a dry lipid film in a solution containing the DNA. Figure 1 A exemplifies the fluorescence images of individual

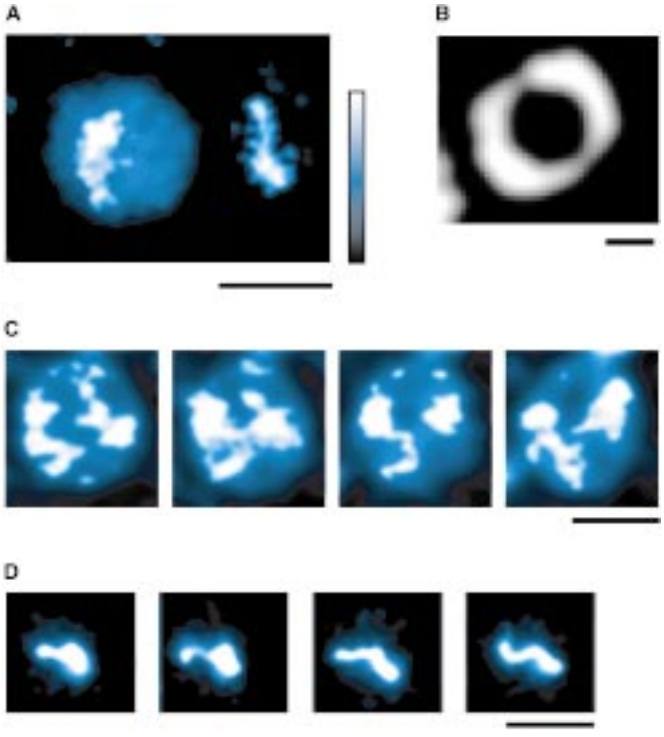


Figure 1. *A: Fluorescence microscopic images of T4 DNA molecules with unfolded coil conformation (stained with DAPI) inside (left) and outside (right) of a giant vesicle of* ***1 a****. The bar represents 5 μm. B: Dark-field microscopic image of giant vesicle of* ***1 a****. In this case, DNA molecules are not visible by dark-field microscopy because of the weak light scattering of the coiled DNA. The bar represents 5 μm. C: Time series of fluorescence images of two coiled DNAs encapsulated in a vesicle: The images were obtained by an image processing (subtraction of time-averaged image from the original images).[5] Time interval between the pictures: 1 s. Brownian motion of the elongated coil DNAs was observed. The bar represents 5 μm. D: Time series of images of a single T4 DNA in the elongated-coil state outside vesicles. Time interval between the pictures: 1 s. The black bar represents 5 μm. The color bar indicates the fluorescence intensity.*

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duplex T4 DNAs stained with a dye (4′,6-diamidino-2-phenylindole, DAPI), inside and outside the vesicles. More than 35% of the giant vesicles (diameter: 2 – 20 μm) entrapped one or several DNAs. Observation of the same sample by dark-field microscopy (Figure 1B) showed that most of the vesicles have an internal volume that is large enough to allow Brownian motion of the entrapped DNAs. Figure 1C shows a time series of fluorescence microscopy images of DNA molecules incorporated into a giant vesicle made up of **1a**. The Brownian motion of the entrapped DNA molecules was clearly observed by image processing[5] (Figure 1C). The DNA molecules are encapsulated in the internal vesicular space, without binding to the vesicle surface. Due to a blurring effect, the actual size of DNA is smaller by ca. 0.3 μm than that observed by microscopy.[3b] The average long-axis length[3] for entrapped DNA molecules (Figure 1C) was 2.7 ± 0.4 μm, which is essentially the same as that of T4 DNA molecules placed outside vesicles (3.1 ± 0.5 μm in this experiment; 2.8 ± 0.7 μm according to ref. [3c]) (Figure 1D). We have extensively studied the folding/unfolding transition between the elongated and the collapsed conformations of DNA in solution.[3] These results show that the encapsulated DNA molecules assume an elongated conformation. It is known that the charged layers in a lamellar liquid crystal expand and swell indefinitely in pure water.[6] It can be understood that, during the natural swelling process, DNAs in bulk solution permeate into the expanding space and are then enclosed in the giant vesicles.

To confirm the entrapment of DNAs within the vesicle, we performed confocal microscopic observation with a double-fluorescence labeling method. Figure 2A shows a fluorescence image of the lipidic membrane stained with BODIPY (far red fluorescence), while Figure 2B shows the corresponding image of DNA molecules stained with YOYO-1 (green fluorescence). Here, we present an optical section (ca. 0.4 μm) for a region dense in vesicles to benefit from the slowing-down effect on the Brownian motion of the vesicles. Thus, it becomes possible to record the same optical section of the vesicles sequentially in the red and in the green channels to obtain these two images. They showed that the movement of DNAs is restricted to the interior of the closed vesicles.

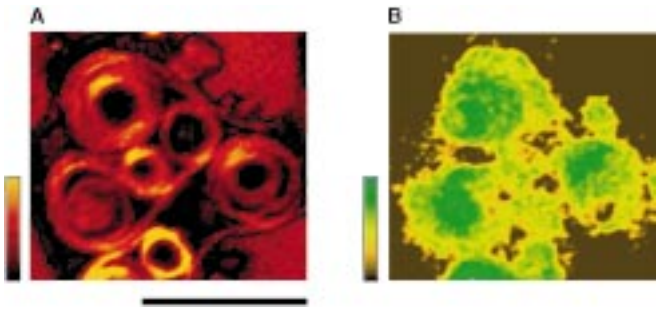

Figure 2. *Confocal microscopic images of DNA molecules encapsulated in giant vesicles of* ***1a****, obtained by a double-fluorescence staining method. A: Image in pseudocolor red of the lipidic membranes stained with BODIPY. B: Image in pseudocolor green of DNA molecules stained with YOYO-1. Scanning time of the confocal microscope is 4 s per image. We selected fluorescence images of aggregated multilameller vesicles. Since the individual entrapped DNA molecules undergo Brownian motion, the fluorescence image of DNA molecules spread out in the internal vesicular spaces. The bar represents 5 μm. The color bars are fluorescence intensities.*

Next, we tried to prepare a giant vesicle that encapsulates folded compact DNAs. We chose histone H1 as an efficient condensing agent for DNAs.[7] Figure 3A depicts a vesicle entrapping a compact DNA; the vesicle was made from **1a** by using the same swelling method as described above. The translational Brownian motion is rather fast for compact DNAs both inside (Figure 3B) and outside of the vesicle (Figure 3C), indicating that they are actually folded into a compact packed state.[8] This is in contrast to the slow motion for coiled DNAs, as seen in Figure 1C.

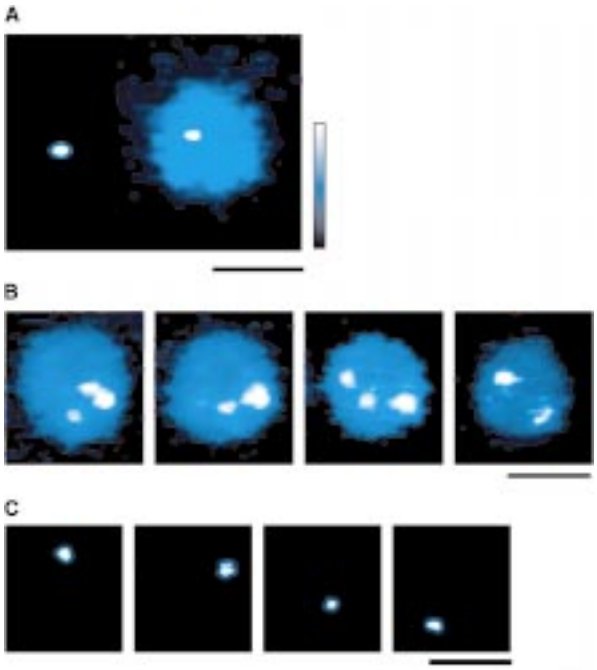

Figure 3. *A: Fluorescence microscopic images of folded compact T4 DNAs complexed with histone H1 (stained with DAPI) outside (left) and inside (right) a giant vesicle of* ***1a****. The bar represents 5 μm. B: Time series of fluorescence images of three encapsulated DNA molecules complexed with histone H1. Time interval between the pictures: 1 s. The Brownian motion of compact DNA complexes was clearly observed. The bar represents 5 μm. C: Time series of the images of a T4 DNA complexed with histone H1 in the condensed state outside vesicles. Time interval between the pictures: 1 s. The black bar represents 5 μm. The color bar indicates the fluorescence intensity.*

In addition to the spontaneous trapping of DNAs into giant vesicles, it would also be desirable to develop a method for incorporating individual DNA molecules into preformed vesicles. Recently, it has been reported that laser trapping is applicable to individual folded DNA molecules.[8] Figure 4 shows the transfer process of the DNA – H1 complex by trapping one complex with a laser tweezer (Figure 4a), and forcibly pushing it from the outside medium into the internal vesicular space of the giant vesicles made of **1b** (Figures 4b and c). Due to elastic stress, the vesicles are deformed (Figure 4c), and finally enclosed (Figure 4d). After the laser has been turned off, the entrapped

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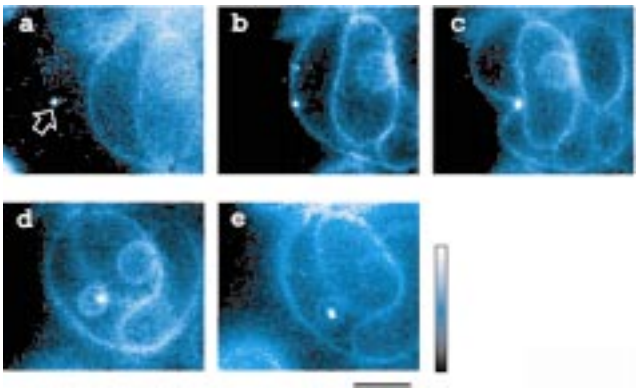

Figure 4. *Fluorescence microscopic images of the optical trapping and transport of a single folded T4 DNA complexed with histone H1 (stained with DAPI) into a giant vesicle. a: Trapping of a T4 DNA – histone H1 complex (arrow); b: transport of the complex to the surface of a vesicle; c: forced transport of the complex into the vesicle and detachment; d – e: entrapment of the complex and its Brownian motion in an inner vesicle, after the laser had been turned off. The black bar represents 10 μm. The color bar indicates the fluorescence intensity.*

DNA continues to exhibit Brownian motion within the vesicle (Figure 4e).

In conclusion, novel methods were used to prepare a model proto-cell that entrapped giant DNA. Studies of such proto-cells would be promising for the future development of research on the origins of life.

Experimental Section

Sample preparations:

Geranylgeranyl phosphate solution for fluorescence microscopy: Geranylgeranyl phosphate **1a** (1 mM) or **1b** (1 mM) in methanol/chloroform (1:2, v/v);[4c]

T4 DNA solution for fluorescence microscopy: Bacteriophage T4 DNA (166 kb; Sigma) was dissolved in HEPES buffer solution (10 mM, pH 7.0). The final concentrations in the DNA solution were: T4 DNA = 0.3 μM (in nucleotide units), DAPI = 0.3 μM ($\lambda_{abs} = 358$ nm/$\lambda_{em} =$ 461 nm; Wako Pure Chemicals).

Geranylgeranyl phosphate solution for confocal microscopy: Geranylgeranyl phosphate **1a** (24 mM) in methanol/chloroform (1:1, v/v) containing BODIPY (0.24 mM, $\lambda_{abs} = 665$ nm/$\lambda_{em} = 676$ nm; Molecular Probes).

T4 DNA solution for confocal microscopy: Final concentrations in HEPES buffer solution (10 mM, pH 7.0) were: T4 DNA = 10 μM (in nucleotide units), YOYO-1 = 0.16 μM ($\lambda_{abs} = 491$ nm/$\lambda_{em} = 509$ nm; Molecular Probes).

T4 DNA – histone H1 complex solution: Complexes of calf thymus histone H1 (30 μM in lysine base; Sigma Type III-ss; lysine-rich histone containing 24.7 wt% lysine and 1.9 wt% arginine) and T4 DNA (30 μM in nucleotide units) were dissolved in NaCl (2 M)/Tris-HCl (10 mM) solution (pH 7.4). Next, the resulting solution was slowly dispersed into pure water to obtain a 0.2 M NaCl solution. The final DNA and histone H1 concentrations were, respectively, 3 μM in nucleotide units and 3 μM in lysine base, which corresponds to a 1:1 charge ratio of DNA to H1.

Fluorescence microscopy: An aliquot (10 μL) of the geranylgeranyl phosphate solution was dropped on a cover glass (0.17 mm thick). After 10 min of drying, 20 μL of the T4 DNA solution or the T4 DNA – histone H1 complex solution were added at 20 °C to the lamellar solid remaining on the slide. Vesicles with a size of 2 – 20 μm formed spontaneously. The sample was observed by fluorescence microscopy (Nikon TE-300, equipped with a Hamamatsu Photonics C2400 – 08 TV camera). The images were recorded on an S-VHS videotape at one frame per 1/30 s. The apparent conformation and spatial position of the individual DNA molecules on the video frames were calibrated with an image processor (Argus 20, Hamamatsu Photonics).

Confocal microscopy: Samples for confocal microscopy were prepared by the same procedure as described in the fluorescence microscopy section. Inverted confocal microscope (LSM Invert 410, Plan Apo x63 objective, Carl Zeiss), laser sources Ar (488 nm, bandpass 515—525-nm filter) and He/Ne (633 nm, longpass 665-nm filter), image processor (Matrox 4MW), PC 486 – 488PX microcomputer, color monitor (FT 3420 ETKL, Mitsubishi), and digital image recorder (Focus).

Laser manipulation: Samples for laser tweezer experiments were prepared by the same procedure as described in the fluorescence microscopy section. The trapping and transport of individual T4 DNA – histone H1 complexes were performed by using a Nd^{3+}:YAG laser (Spectron, SL-902T) at $\lambda = 1064$ nm with a power of 250 mW.[8]

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