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Study of ternary complex (TC): DNA-PC liposomes-Mg^{2+} as base for nuclear pore assembly by relaxation time fluorescence microscopy.

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［2］ Research Progress

This stage of project related to choice of model system: ternary complex located inside giant liposome (GUV containing inside DNA and LUV 100 nm in diameter) Addition to lipids ionophore A23187 for Mg^{2+} produce pores in GUV for addition of Mg^{2+} or Ca^{2+} inside GUV

［3］ Results

Try to get a TC with the unwinding of the DNA using a method of DNA induced by the fusion of TC with liposomes with a diameter about 1 μm. The resulting liposomes have a diameter greater than 1 μm may be examined in detail in the new microscope. To investigate such TC is convenient if they are located in a small volume (several μm^3). This can be done microcuvette, but in this case it is inconvenient to add any substance. Easier to work with conventional cuvette volume of 300 μl, the type used in the laboratory Prof. M. Yamazaki [1] and TC put into giant liposomes with holes for the cations Ca or Mg, produced by ionophore A23187. The scheme of experiment is shown in Fig.1.

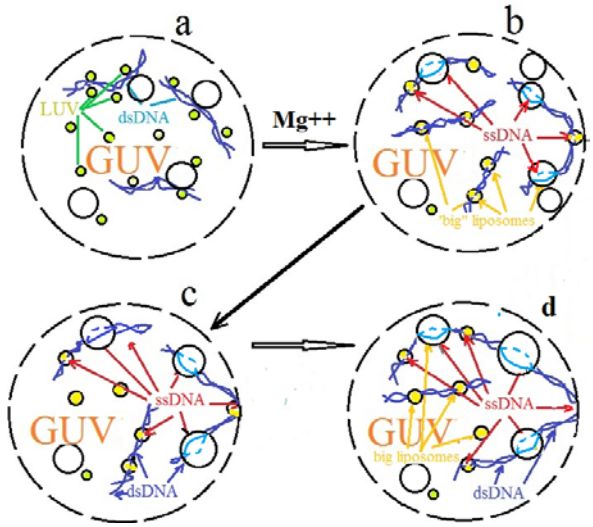

Fig.1. Scheme of proposed experiments: a) dsDNA and LUV inside GUV in 10 mM HEPES buffer, ph7.5; green circles-LUV (100nm in diameter); b) after addition of 2 mM Mg++ forming ternary complexes (TC-yellow circles) interact with similar size LUV creating LUV (up 1-5 μm in diameter); c) and d) interaction of TC with GUV with transfer ss DNA on the surface of GUV.

Reagents and methods.

Calf thymus DNA, DNaseI was from (Sigma-Aldrich, USA), $MgCl_2*6H_20$, $CaCl_2$ (Waco, Japan), DOPC and DOPA was from Avanty polar lipids, (USA); sucrose, glucose, NaCl, obtained from Wako (Japan).

Liposomes (LUV) from DOPC/DOPA (50/50 mol%) were got with the multilamellar liposome extrusion in 0.01M HEPES buffer, pH-7.5. The GUV with the same lipid composition was prepared according to the method, described in [2]. Spectroscopic studies were conducted with the help of Hitachi spectrophotometer. Fluorescence of such probes as CF, calcein, EB was analyzed with the help of a spectrofluorimeter UV-1800 Shimadzu, Japan, spectra of fluorescence obtained by spectrofluorimeter Hitachi F-4500, Japan. Samples of GUV was analyzed in fluorescence microscope Olympus (IX-70, IX-71) Tokyo, Japan. Presented data made minimum in 3 measurements, error of measurement was less 10%.

(３－１) Research results

The first thing that catches the eye is the fact that GUV1 contain less liposomes with the size of 20 □40 □m in the presence of 1mM Mg2+. In this case, the part compared to the control sample (where there are no additives inside GUV). In our opinion this is due to fact, that the additives, which are present in the HEPES buffer, initiate formation of liposomes (LUV), some of which are captured by large GUV1. The increased number of multilamellar liposomes can be explained by this theory. But the most interesting thing is the substantial activity in GUV1 and especially inside multilamellar liposomes even when there are no divalent cations. It is both the movement of LUV (1-5 μm) in GUV1 and their fusion (increase in the liposome size). In our opinion it is the evidence DNA interaction with LUV and GUV1 in the absence of Mg 2+. Previously we have shown that such interaction is possible in the case TC of zwitterion liposomes (DMPC) studied by DSC [3]. There is other evidence of such interactions [4]. Unfortunately, there is little information about the nature of such interactions. However, we can assume that the nature of the DNA interaction with LUV and GUV in the presence or absence of Mg 2+ is significantly different. If we add only 1mM Mg2+ to GUV1, a rapid process of restructuring from one type of complexes to the other is begin.

It is followed by the movement of LUV inside GUV, and GUV itself, and also the LUV fusion and it finishes at 50% of the cases by burst of GUV with the release of LUV, connected together with DNA strands (Fig. 2).

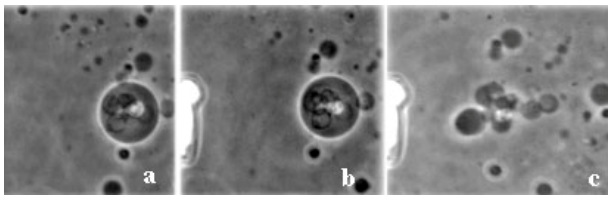

Fig. 2. Addition with help micropipette of 1mM of Mg^{2+} to GUV containing LUV+DNA inside results in moving and fusion of LUVs with burst of the GUV and LUV binding by DNA come out.

Now let's try to understand what has caused the movement of liposomes in the GUV 1 (containing DNA + LUV) after we have added 1mM Mg^{2+}. As it was shown in [5], only 20% of the added DNA is associated with the liposomes in the absence of Mg^{2+} or Ca^{2+} cations. The remaining 80% of a free DNA can form TC from LUV. In this case, the part of the DNA previously associated with lipids may lose this connection and also form a TC. This will undoubtedly cause the LUV movement inside GUV and their fusion up to micron size, visible in the phase-contrast microscope (Fig3). If we add DNaseI to GUV1 after the LUV movement stops the LUV movement will resume again (Fig.3).

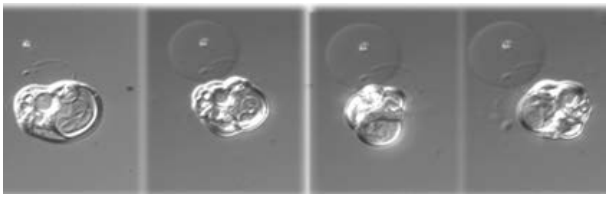

Fig.3. Moving and fusing of LUV inside GUV1 after addition of DNaseI (time between frames is 30 sec.

It can be explained by the fact that the DNA forming TC inside GUV1 makes a lot of links among various liposomes. Many of these are in a strained state; the DNA is supercoiled and thereby compensates for the excess of interliposome link tension. Therefore, due to digestion of DNA by DNaseI, strain energy is gradually released; it results in stepwise LUV rotation and movement inside GUV1. We believe that DNaseI penetrates GUV1 through the pores which are formed by Mg2+ and LUV.

We believe that the formation of TC result in significantly changing the curvature of the liposomes and this system is suitable for the study using relaxation time fluorescence microscopy.

References

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