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Metaphase chromosomes from mammalian cells stimulate fusion of artificial phospholipid vesicles

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Isolated mitotic chromosomes are able to form complexes with phosphatidylcholine liposomes in the presence and absence of Ca^{2+} ions, in the latter case in the presence of polyamines. Interactions with chromosomes stimulates liposome fusion. The fusion is promoted by condensed and EDTA-decondensed chromosomes.

Chromosome; Liposome; Resonance energy transfer; Fusion

1. INTRODUCTION

Mechanism of the nuclear envelope assembly at the end of mitosis is still unclear. It is well known that in the late telophase membrane vesicles are adsorbed on chromosomes and fused. Obviously this should be preceded by specific binding of vesicle-precursors to the chromosomes and induction of vesicular fusion. Experiments with fractionated mitotic extracts of *Xenopus laevis* showed that about 20% of vesicular material could interact with sperm chromatin and form the nuclear envelope [1]. Preliminary trypsin treatment of the vesicles hindered these processes. These data proved the existence of a protein factor providing selective binding of the vesicles to the chromosomes or fusion of the vesicles bound to the chromosomes.

The object of this study was to investigate one of the steps of nuclear envelope assembly, a process of vesicular fusion. We made use of a model system consisting of isolated mitotic chromosomes from mouse fibroblasts and phosphatidylcholine liposomes as analogs of vesicle-precursors of the nuclear envelope. It was demonstrated that the interaction of chromosomes with liposomes caused fusion of membranes. The factor inducing the fusion of vesicles is an integral element of chromosomes.

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Abbreviations: DPPC, [^{14}C]dipalmithoylphosphatidylcholine; PC, phosphatidylcholine; NBD-DPPE, *N*-(4-nitrobenz-2-oxa-1,3-diazole-7-yl)-dipalmithoylphosphatidylethanolamine; Rhd-DPPE, *N*-(rhodamine B)-dipalmithoylphosphatidylethanolamine; TLC, thin-layer chromatography; Tris, Tris(hydroxyethyl)aminomethane; EDTA, ethylendiaminetetraacetic acid; RET, resonance energy transfer

2. MATERIALS AND METHODS

2.1. *Materials*

The following chemicals were used: T7 DNA (Sigma, USA), [^{14}C]dipalmithoylphosphatidylcholine (DPPC) with activity of 115 mCi/mmol (Amersham, UK), β-octyl-D-glucopyranoside (Fluka, Switzerland). Phosphatidylcholine (PC) from egg yolk was isolated by standard procedure [2]. Fluorescent derivatives of phospholipids – NBD-dipalmithoylphosphatidylethanolamine (NBD-DPPE) and (rhodamine B)-dipalmithoylphosphatidylethanolamine (Rhd-DPPE) – were synthesized from 1,2-dipalmithoylglycero-3-phospatidylethanolamine (Fluka, Switzerland) and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (Sigma, USA) or rhodamine B isothiocyanate (Sigma, USA), respectively, as described in [3].

2.2. *Isolation and purification of chromosomes*

Chromosomes were isolated from cultured mouse fibroblasts (line A9). Cells were synchronized by the addition of colchicine (0.2 μg/ml) for 12–16 h. Mitotic cells were removed by shaking. Isolation of chromosomes was performed by standard methods: either in the presence of $CaCl_2$ [4] or in buffer containing polyamines as stabilizing agents [5]. Isolated chromosomes were treated with 1% octylglucoside followed by centrifugation through 0.3 M sucrose on conventional buffer at 4000 × *g*, 15 min. The level of contamination of the chromosomal preparations by membrane material was estimated by TLC after extraction of lipid using a mixture of chloroform/methanol/water (1:2:0.6).

2.3. *Preparation of the liposomes*

To prepare small unilamellar liposomes a mixture of PC/NBD-DPPE/Rhd-DPPE in a 100:0.5:0.5 molar ratio was evaporated and sonicated in ice in 10 mM Tris-HCl, pH 7.5 [^{14}C]DPPC-labelled liposomes were obtained by sonication of a mixture of PC/[^{14}C]DPPC (3.6 μCi/mmol of lipid). After sonication large liposomes were removed by centrifugation at 10 000 × *g*, 15 min.

Uranyl acetate-contrasted liposomes were examined on an EM-1000C electron microscope. The fluorescence was measured on a Hitachi MPF-4 fluorescence spectrophotometer.

3. RESULTS AND DISCUSSION

Binding of chromosomes to liposomes is a necessary prerequisite of vesicular fusion.

Earlier we demonstrated that DNA and chromatin are able to form complexes with the liposomes in the presence of divalent cations [6]. The formation of these complexes is based on the interaction of Ca^{2+}-ions with phosphate groups of both DNA and phospholipids. Chromosomes can also couple with liposomes in the presence of divalent cations [7]. The formation of these complexes is accompanied by chromosome decondensation and migration of a 33 kDa chromosomal protein to the liposomes. Addition of EDTA dissociates the complex, promotes decondensation of the chromosomes and leads to a release of several chromosomal proteins [7]. Nevertheless chromosomes can be isolated without Ca^{2+}-ions but in the presence of polyamines (PA chromosomes).

At first we studied the ability of the chromosomes isolated in the presence of polyamines to liposome binding. Incubation of the PA chromosomes with liposomes in the presence of 0.35 mM of spermidine results in adsorption of the liposomes on the chromosomes. Complex formation was detected by co-precipitation of the [^{14}]DPPC-labelled liposomes with the chromosomes in the presence of spermidine. It is noteworthy that under the same conditions free DNA does not accept liposomes but binds them effectively in the presence of divalent cations. At 1 A_{260}/ml DNA concentration and 0.125 μM/ml lipid concentration, PA chromosomes bind 15 nmol of lipid per 1 A_{260} of DNA which is much less than Ca^{2+}-stimulated adsorption (68 nmol of lipid per DNA unit). The repeated centrifugation in fresh buffer induces 57% dissociation of the complexes. The addition of Ca^{2+}-ions in 3 mM concentration to the same mixture stimulates further binding of liposomes up to 45 nmol of lipid per 1 A_{260} of DNA.

Using light microscopy it was shown that the formation of Ca^{2+}-independent chromosome/liposome complex did not alter chromosome morphology.

Fusion of artificial vesicles interacting with chromosomes was estimated using the resonance energy

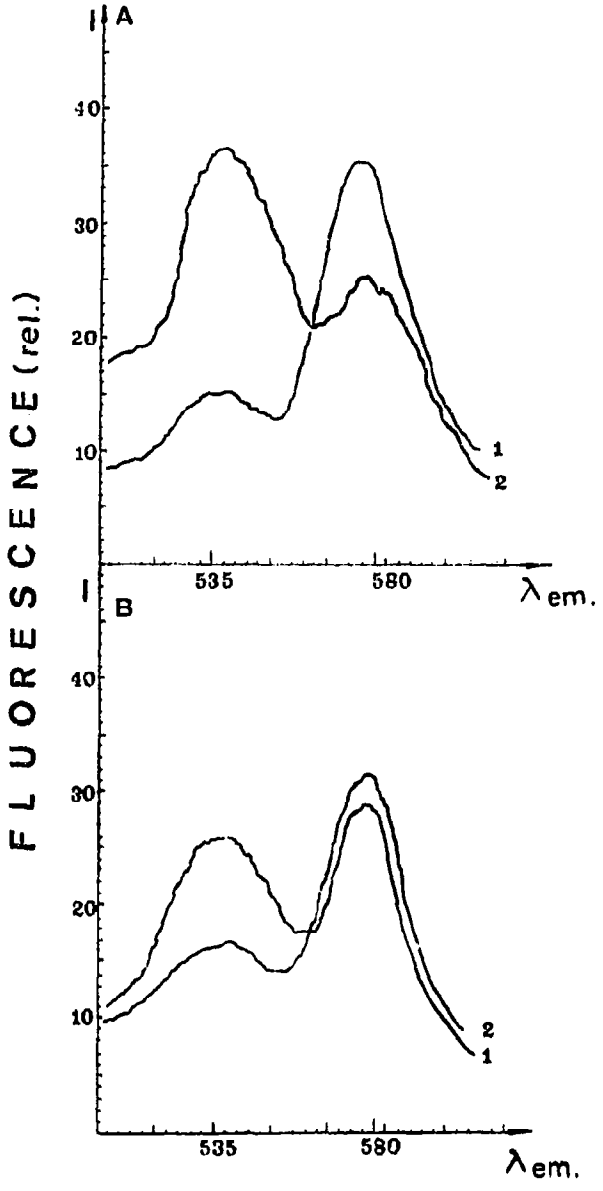

Fig. 1. Fluorescent spectra of the liposomes. (A) 1 = spectrum of initial liposomes (it is identical to the spectrum of unbound liposomes); 2 = spectra of the liposomes released from the complexes with the chromosomes by EDTA treatment. (B) 1 = is the same as in (A); 2 = spectra of the liposomes released from the complexes with PA chromosomes. Liposomes were excited at 468 nm that corresponds to the maximum of the excitation for donor of energy (NBD-DPPE).

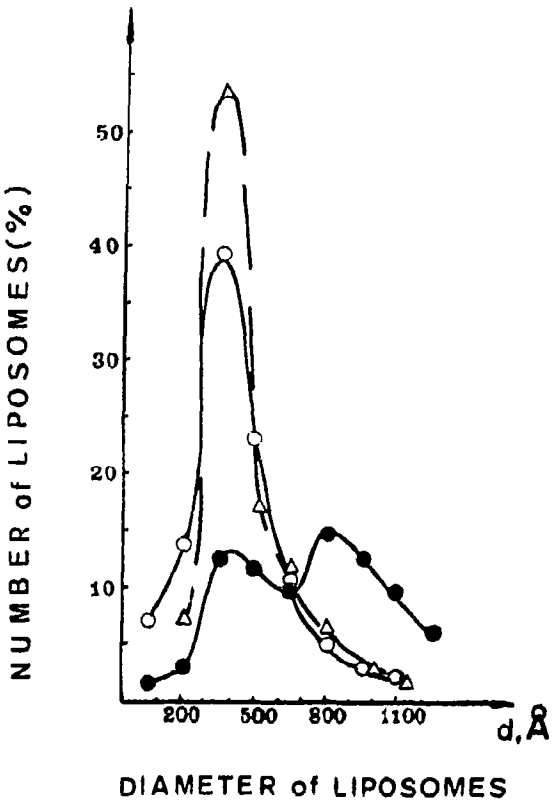

Fig. 2. Size distribution of vesicles (according to electron microscopy). (○) Initial liposomes; (△) unbound liposomes; (●) vesicles released from the complexes with chromosomes by EDTA. In each fraction 200 liposomes were measured.

transfer (RET) from one fluorescent analog of lipids, NBD-DPPE (donor), to another, Rhd-DPPE (acceptor of energy) [3]. Chromosomes were incubated with a mixture of fluorescent-labelled liposomes and 10-fold excess of unlabelled liposomes in the presence of Ca^{2+}-ions. The complexes were precipitated to remove unbound liposomes. Then the complexes were dissociated by EDTA followed by resedimentation. The resultant supernatant contained liposomes released from the complexes with the chromosomes. As shown in Fig. 1A, spectral characteristics of these liposomes are quite different from those of the initial liposomes and correspond to the spectra of fused liposomes. The incubation of the liposomes with DNA, normally resulting in the formation of complexes, did not lead to fusion of the vesicles (data not shown).

Electron microscopy of the vesicles released from the complexes with chromosomes revealed two types of liposomes. The average size of the first type is 360 Å which corresponds to the diameter of the initial liposomes and the unbound liposomes. In contrast, the vesicles of the second type are much larger – about 800 Å on the average (Fig. 2).

So a significant level of fusion was observed for the liposomes dissociated from the complexes with the chromosomes.

It has been mentioned above that Ca^{2+}-dependent adsorption of the liposomes induces chromosomal decondensation. The decondensation is also caused by EDTA treatment, then it is accompanied by the release of chromosomal proteins in solution [7]. This means that decondensed chromosomes or released chromosomal proteins may have fusogenic activity. For identification of a chromosomal fraction responsible for fusogeneity EDTA-treated chromosomes were separated from the released proteins by centrifugation. The solution of the latter did not stimulate fusion of the vesicles, unlike decondensed chromosomes which did, this fact being proved by RET.

The liposomal fusion was detected in Ca^{2+}-independent complexes with chromosomes. The complexes were obtained by centrifugation as described previously. The liposomes were released under recentrifugation of complexes. The spectra of the released liposomes clearly demonstrated their fusion (Fig. 1B). Since there was no decondensation in the case of Ca^{2+}-independent complexes, we concluded that it is not necessary for the fusion to start. Our data suggest that the fusogenic activity required for the formation of the nuclear envelope from vesicle-precursors is localized in the chromosomes and is not destroyed even by EDTA-treatment. Ca^{2+}-ions and decondensation of the chromosomes are not necessary for fusion either. It seems therefore that the duty of the protein factor described in [1] must be the adsorption of the vesicle-precursors on the chromosomal surface while the fusion of the adsorbed membranes is guided by structural elements of the chromosomes.

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