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DNA-bound lipids of normal and tumor cells: retrospective and outlooks for functional genomics

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Abstract

By very soft phenol method, the high-molecular-mass natural DNA complexes (10^8–10^9 Da), which contain 1–3% specific lipids, were isolated from different eukaryotic and prokaryotic cells. Two pools of DNA-bound lipids were isolated: loosely bound (extracted with 35% ethanol) and tightly bound lipids (extracted after additional treatment DNAse I). The composition of these two lipid pools of different sources (rat thymus, liver, regenerating liver, loach sperm, pigeon erythrocytes, Zajdel ascites hepatoma, Ehrlich ascites carcinoma, sarcoma 37, *Escherichia coli* B, T2 phage) was studied. The DNA-bound lipid pools consist of neutral lipids (NL) and phospholipids (PL), moreover NL is always in a few fold more than PL. The composition of these lipid pools of eukaryotes distinguishes between themselves, mainly, by free cholesterol (minor fraction), cardiolipin (major fraction), and by phosphatidylcholine. Only the tightly bound lipid pool was present in T2 phage DNA. The dramatic redistribution effect between all fractions of NL pools (free and ester cholesterol, free fatty acids, diglycerides) was observed in DNA synthesis phase of cell cycle on the background of the unchanged composition of PL pools. Comparative analysis of DNA-bound lipid pools of normal and cancer cells was carried out. The DNA-bound lipid pools of transformed cells significantly differ from the same normal cells both by PL composition (cardiolipin) and by the presence of additional fractions (mono- and triglycerides) as well. The possible functions of DNA-bound lipid pools, especially of cardiolipin and cholesterol at the attachment of DNA loops to the nuclear matrix, DNA replicon organization, replication, and transcription are discussed.

Keywords: Two pools of DNA-bound lipids; Lipid composition and function; DNA-bound cardiolipin; DNA-bound cholesterol; Functional genomics

1. Introduction

Up to now, it has been a widespread opinion that only proteins can participate in structural–functional organization of DNA. However, owing to the progress in lipidology for the past 10–15 years, it has been proven that lipids are actively involved into chromatin and nucleosome structure [1–3], in regulation of genome expression, into the replication, transcription and repair processes [4–8], into the induction of apoptosis [9–12], and appear to be a target for ionizing radiation and antitumor preparations [13]. Along with development of gene therapy methods, the problem of plasmid DNA transfer into the cells (reporter or therapeutic genes) arised [14,15]. For this purpose, various lipids and their mixtures are used in *in vitro* experiments to obtain artificial macromolecular DNA–lipid complexes, i.e. genosomes [15]. Clearly, at the present stage of gene therapy and functional genomics as well, progress in the problem of isolation and characterization of natural DNA–lipid complexes is a question of special importance. It is not accidental that natural high-molecular mass DNA–lipid complexes (1–2% of protector lipids) have very high transforming (gene transfer) activity (50%) as it was shown in studies on chicken (the complexes obtained from the dominant Russian white were introduced into the egg of New Hampshire [16]).

The natural DNA-bound lipid complexes were first isolated by us from rat liver and thymus in 1974 [17], then they were isolated from other mammalian cells, tumor cells, bacteria, and phage [18–21]. The isolation and general characterization of DNA-bound lipids are described in

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details in Ref. [22]. We should point out that DNA-bound lipids have specific composition in mitochondria, microsomes, nuclear membranes, chromatin, and nuclear matrix. The formers are characterized by prevalence of neutral lipids (NL) over phospholipids (PL), high enrichment in cardiolipin, cholesterol fatty acid esters, free fatty acids, and diglycerides but depletion of free cholesterol, phosphatidylethanolamine, phosphatidylcholine—while having only trace amounts of sphingomyelin. Besides, these lipids consist of two pools differing in degree of their binding to DNA and in their composition [17,21–23]. It was proved in special experiments that DNA-bound lipids are an integral part of DNA and not an artifact of DNA isolation procedure, e.g. lipid admixtures [21–23].

This review is aimed to summarize and to analyze critically our own and literature data on the composition and possible functions of the two pools of DNA-bound lipids of eukaryotes and prokaryotes, and to undertake a comparative analysis of these lipid pools in normal and malignant cells. Our goal is to demonstrate that natural DNA-bound lipids are an integral part of genome taking part in regulation of gene expression as well.

2. Historical aspects

In reviewing the history of the problem, it should be noted that the first observation of the presence of lipids in calf thymus nucleohistone was made in 1959 by Wilkins and Zubay [24] based on the small-angle X-ray diffraction. Specifically, two reflexes were found on nucleohistone roentgenogrammes: the first one at 20 Å (DNA) and the other one at 60 Å (lipids). This conception was only tentative since a similar picture was observed with 3% sphingomyelin, which also gave a highly oriented equatorial reflex at 60 and 15 Å. In 1972, Pardon and Wilkins [25] confirmed their earlier reported data. In 1966, we have also observed a second reflex at 60 Å for high-molecular mass DNA from loach sperm and rat thymus [26]. However, at that time, no data on DNA-bound lipids were available; in view of that, only in 1993, it became possible to demonstrate directly that the reflex at 60 Å corresponds to DNA-bound lipids.

It is to be noted that until 1974, the data on the presence of chromatin- and DNA-bound lipids were scarce and fragmentary. Rose and Frenster [27] were the first (1965) to reveal lipids in eu- and heterochromatin of calf thymus and to estimate their amount. Lipids contained ~70% phosphatidylcholine, ~30% phosphatidylethanolamine, 35% cardiolipin, traces of phosphatidylserine and sphingomyelin (percentage of the total amount of phospholipids), and 97% of free cholesterol (percentage of the total amount of neutral lipids). Jackson et al. [28] have also found lipids in the insoluble precipitate of calf thymus chromatin (30.1 mg proteins, 9.0 mg lipids, 2.1 mg DNA, 0.4 mg RNA), namely phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, and unidentified neutral lipids.

As it was mentioned, we isolated the natural DNA-bound lipids from rat liver and thymus for the first time in 1974 [17]. These lipids consisted of two pools differing in tightness of their binding to DNA. The loosely bound pool of lipids is extracted with ethanol (DNA is precipitated by cold 96% ethanol (1:2) and is allowed to stay at 2 °C for 24 h, and then lipids are extracted by chloroform–methanol mixture (2:1) using the Folch's method [29]). The tightly bound lipid pool (DNA precipitate after ethanol treatment) was dissolved in 1% SDS solution, and then the lipids were again extracted using the Folch's method. This procedure allows us to prepare the DNA-bound lipid pools consisting of only neutral lipids, with phospholipids in trace amounts, which suggests the incomplete extraction of lipids (especially of

Table 1
Comparative analysis of contents of DNA-bound lipids in DNA preparations from different sources by various methods

Object [reference]	Method of DNA isolation [reference]	DNA molecular mass (Da)	DNA-bound lipids (% in DNA)		
			Total	Neutral lipids	Phospholipids
Rat liver [21]	[34]	29×10^7	2.1±0.2 (1.19+0.91)	1.49±0.12 (0.86+0.63)	0.61±0.04 (0.33+0.28)
Ehrlich ascites carcinoma [21]	[34]	28×10^7	2.23±0.21 (1.36+0.87)	1.69±013 (1.03+0.66)	0.54±0.03 (0.33+0.21)
Zejdel ascites hepatoma [21]	[34]	28.5×10^7	2.97±0.25 (1.76+1.21)	2.52±0.22 (1.47+1.05)	0.45±0.02 (0.29+0.16)
E. coli B. [21]	[34]	20.5×10^7	0.89±0.08 (0.58+0.31)	0.78±0.07 (0.53+0.25)	1.11±0.01 (1.05+0.06)
Mouse liver [33]	[35]	14×10^6	1.0	Not identified	PC, SM
Ehrlich ascite carcinoma [33]	[35]	15×10^6	3.0	Not identified	PC, SM
S. typhimurium [31]	[35]	20×10^6 15×10^6	0.17±0.04	Diglycerides, fatty acids, triglycerides	Not identified
Ehrlich ascites carcinoma [32]	5% Cl_3CCOOH 80 °C, from chromatin, pretreated with triton X-100	–	0.89	0.87	0.02

Marmur's method [35]: 1% SDS, 1 M NaCl, chloroform/iso-amyl alcohol=24:1, RNase, pronase, phenol. In parenthesis: (loosely bound lipids +tightly bound ones); PC: phosphatidylcholine, SM: spingomyelin.

tightly bound ones) from DNA. In view of this, the method was subsequently modified: to extract the 1st pool (loosely bound lipids), we used 35% ethanol at 37 °C for 24 h (DNA would not precipitate under this conditions, which facilitates extraction of lipids); and to extract the 2nd pool, we employed the incubation with DNAse I (2 h, 37 °C) instead of using 1% SDS. Every pool of DNA-bound lipids consists of neutral lipids and phospholipids. As it was shown in our subsequent studies [18–21], this procedure was optimal, since it allows to follow changes and redistribution of lipid pools depending on the cell cycle, genome activity, alterations in DNA conformation, and under malignancy.

To our knowledge, there are only three studies [31–33] where lipid content was determined directly in DNA. As it is shown in Table 1, the DNA preparations, isolated from various sources (Ehrlich ascites carcinoma, Zajdel ascites hepatoma, rat liver, mouse liver, *Escherichia coli* B, *Salmonella typhimurium*) and by various methods [34,35], contain statistically significant amounts of tightly bound lipids. It shoud be noted that two DNA preparations from Ehrlich ascites carcinoma, prepared by various isolation techniques (a gentle or hard one [34,35]), contain essentially the same lipid percentage (2.23% and 3%, respectively). Under application of even harder method for DNA isolation from the certain object (at first, Triton X-100 extraction of histones and nonhistone proteins from chromatin and then isolation of DNA itself by use of 5% perchloroacetic acid (PCA), at 80 °C for 20 min), the DNA preparation contains a less amount of tightly bound lipids (0.89%) and the specific composition (the amount of neutral lipids was 40-fold higher than that of phospholipids) [32]. It was shown further [31] that lipid content in the DNA preparation from *S. typhimurium* depends on the DNA molecular mass, i.e. the higher molecular mass DNA contains, the greater amount of lipids. For instance, the DNA preparations, isolated by various techniques from *E. coli* B [34] and *S. typhimurium* [35], contain 0.31% [21] and 0.17% [31] of tightly bound lipids, respectively. Thus, DNA preparations, isolated by various authors by the use of methods of more or less hardness from various sources, always contain lipids in the range 0.17–3.0%.

3. Comparative analysis of composition of two pools of DNA-bound lipids from normal and malignant cells, *E. coli* B and T2 phage

Table 2 presents the compositions of two pools of DNA-bound lipids from various eukaryotic cells (normal and malignant), *E. coli* B and T2 phage. The composition of two lipid pools in rat thymus differ from each other for the most part, in amounts of free cholesterol, cardiolipin, and phosphatidylcholine, with free cholesterol being the minor fraction while cardiolipin the major one. Analogous regularity was also demonstrated for rat liver. Characteristically, these lipid pools are enriched in cholesterol fatty acid esters, free fatty acids, diglycerides, cardiolipin, and phosphatidylethanolamine while being depleted in free cholesterol and phosphatidylserine. Besides, the loosely bound pool of phospholipids contained fewer amounts of cardiolipin and free cholesterol than did the tightly bound one.

Table 2
Composition of two pools of DNA-bound lipids (percentage from total neutral or from total phospholipids)

Object	Lipid pool	Neutral lipids						Phospholipids				
		FC	CE	FFA	DG	MG	TG	CL	PE	PC	PS	PI
Rat thymus	A	10	33	33	24	0	0	51	28	15	4	2
	B	13	32	32	23	0	0	55	28	12	4	1
Rat liver:	A	9	36	25	30	0	0	40	35	20	4	1
norm	B	10	34	23	33	0	0	49	28	15	3	5
S-phase	A	6	55	10	29	0	0	39	35	19	5	2
	B	13	46	17	24	0	0	48	28	15	4	5
G_2-phase	A	2	18	57	23	0	0	33	33	27	7	0
	B	8	15	47	30	0	0	15	16	54	15	0
Loach sperma,[a]	A	5	29	41	25	0	0	22	41	28	7	2
at 2 °C	B	6	23	17	54	0	0	48	25	20	7	0
Loach sperm,[b]	A	10	38	9	43	0	0	56	29	12	3	0
at 14 °C	B	18	14	42	36	0	0	47	33	16	4	0
Pigeon	A	4	44	36	16	0	0	19	45	29	7	0
erythrocytes	B	4	39	32	25	0	0	26	35	31	8	0
Zejdel ascites	A	10	33	24	30	0	3	50	26	16	4	4
carcinoma	B	4	46	23	17	7	3	35	40	20	5	0
Ehrlich ascites	A	15	29	22	31	0	3	48	37	12	3	0
carcinoma	B	4	43	24	19	7	3	43	13	28	7	9
Sarcoma 37	A	11	45	22	19	0	3	52	23	18	5	2
	B	20	23	27	26	0	4	33	34	16	5	12
E. coli B	A	3	32	35	30	0	0	71	29	0	0	0
	B	3	45	35	17	0	0	78	22	0	0	0
T2 phage	B	3	37	35	25	0	0	59	41	0	0	0

Mean value from three to four experiments, ±6%.
A: loosely bound lipids, B: tightly bound lipids; FC: free cholesterol, CE: cholesterol fatty acid esters, FFA: free fatty acids, DG: diglycerides, TG: triglycerides, MG: monoglycerides, CL: cardiolipin, PE: phosphatidylethanolamine, PC: phosphatidylcholine, PS: phosphatidylserine, PI: phosphatidylinositol.
[a] Supercoiled DNA.
[b] Relaxed DNA.

The latter peculiarity is also a characteristic for the most repressed genomes (loach sperm, pigeon erythrocytes). Both pools were also characterized by the two-fold lowering of free cholesterol and cardiolipin, as a result, it is the phosphatidylethanolamine fraction that becomes the major one instead of cardiolipin. Besides, both of these genomes are characterized by the lack of phosphatidylinositol.

At the stage of DNA synthesis (S-phase of cell cycle), on the background of equal compositions of phospholipids pools, a dramatic effect of redistribution between the four fractions of neutral lipids pools was observed. For the loosely bound pool of neutral lipids, this effect is represented by a two-fold decrease in free cholesterol and free fatty acids, and at the same time by an increase in cholesterol fatty acid esters, which thus become major. In contrast, the tightly bound pool of lipids is characterized by a sharp increase in free cholesterol and its esters, and a decrease in free fatty acids and diglycerides content.

The effect of redistribution in pool's composition of neutral lipids and phospholipids was also observed in G_2-phase. In both pools of neutral lipids, they observed a sharp decrease of free cholesterol and its esters and an increase by free fatty acids, which become major. The least changes are observed in diglyceride's content. A dramatic decrease of cardiolipin, especially of its tightly bound form along with phosphatidylinositol disappearance, was revealed in both phospholipid pools, at the same time, an increase in the amounts of phosphatidylserine and phosphatidylcholine was registered, with the latter becoming major in the tightly bound pool.

Contrary to the DNA of normal cells, DNA-bound lipid pools of malignant cells are characterized by essential differences between pool compositions both in phospholipids and neutral lipids, and also, by the presence of additional fractions, i.e. by mono- and triglycerides. It is important that these additional fractions were also revealed in the DNA of Ehrlich ascites carcinoma [32]. However, the effect of redistribution between lipid pools has another behavior than in the regenerating liver. This effect was most clearly observed upon comparison of lipid pools of normal liver with those of Zajdel ascites hepatoma. For instance, the amount of loosely bound lipids (cholesterol, diglycerides) in Zajdel hepatoma, in opposite to the DNA of normal liver, is twice higher than the amount of tightly bound one. Moreover, the composition of neutral lipid pools differs significantly from each other. Analogous regularity was met for phospholipid pools. The redistribution effect is especially registered in the tightly bound pool where phosphatidylethanolamine instead of cardiolipin becomes major.

As a rule, in the DNA of two types of malignant cells, in opposite to the DNA of normal cells, the loosely bound free cholesterol occurs in the higher amounts than the tightly bound one; the tightly bound neutral lipids are much poorer in diglycerides and the tightly bound phospholipids—in cardiolipin. Thus, malignant cells have specific composition of DNA-bound lipid pools. This conclusion is confirmed by the data on the main indexes of DNA-bound lipid pools (the ratio of cholesterol ester/free cholesterol, the neutral lipids/phospholipids ratio and percentage of acid phospholipids) (Table 3). It is also shown [36] that the free cholesterol/cholesterol fatty acid esters system in rat liver chromatin is a dynamic system. As it follows from data of Table 3 in the pools of DNA-bound lipids of malignant cells, this dynamic equilibrium is disturbed. Furthermore, the ratio of neutral lipids/phospholipids is significantly increased, especially in the Zajdel ascites hepatoma (by three-fold) compared to the normal liver and to S-phase of cell cycle. Besides, the tightly bound phospholipids in normal cells are more acidic than the loosely bound ones, while for malignant cells, the reverse is true; loosely bound phospholipids appear to be more acidic.

It should be pointed out that two phosphatidylinositol pools are isolated from the mouse and human tissues—a mobile pool (extraction with chloroform–methanol, 2:1) and a tightly bound pool (extraction with chloroform–methanol–1% aqueous HCl solution). Characteristically, the prevalence of the mobile phosphatilylinositol pool is observed in Luis carcinoma mice and in patients with lung cancer [37].

Table 3
Comparative composition of two pools of DNA-bound lipids in normal rat liver, regenerating liver (S-phase and G_2-phase of cell cycle) and in three types of tumor cells

Object	Lipid pool	CE/FC ratio	Neutral/phospholipids ratio	Sum of acidic phospholipids (%)
Normal liver	A	3.82	2.6	45
	B	5.02	2.24	57
S-phase	A	9.53	1.88	46
	B	3.65	3.26	57
G_2-phase	A	8.67	7.14	40
	B	1.97	5.84	50
Zejdel ascites hepatoma	A	3.13	5.1	58
	B	12.48	6.3	40
Ehrlich ascites carcinoma	A	1.99	3.12	51
	B	3.33	3.19	59
Sarcoma 37	A	4.26	3.75	59
	B	1.14	2.11	50

A: loosely bound lipids, B: tightly bound lipids; CE: cholesterol fatty acid esters, FC: free cholesterol.

Thus, malignant cells are characterized by the enhanced content of neutral lipids, in particular, cholesterol and its esters, and by reduced content of phospholipids, particularly cardiolipin and phosphatidylethanolamine. Considerable increase (by two- to three-fold) of precisely neutral lipids in nuclei of Ehrlich ascites carcinoma cells with age (7 and 14-day cells) was observed [38].

The fact of triglycerides' presence in the DNA and chromatin of malignant cells [19,21,32] allows the suggestion to be made that triglycerides are the main form of existence of free fatty acids for the storage of substrates for oxidative phosphorylation.

All these observations pointing to the alterations in regulation of oxidative reactions of lipids in malignant cells lead, as a result, to the alterations in DNA and RNA synthesis. Indeed, based on these data, we have found the effect of recoding of chromatin's gene activity in Ehrlich ascites carcinoma [39]. This recoding effect is expressed by the rates of inclusion of /^{14}C/-thymidine into DNA, and /^{14}C/-uridine into RNA of malignant cells. These values were twice higher in the case of heterochromatin than for euchromatin, while in the normal mouse spleen, the ability of euchromatin to include these two labels was much higher than that of heterochromatin.

The lipid pools in the DNA of *E. coli* B and T2 phage have their own peculiarities: neutral lipids consist of the same four individual lipids like in normal eukaryotic cells, but phospholipids are only presented by cardiolipin (major) and phosphatidylethanolamine. Besides, in the DNA of T2 phage, only the tightly bound lipid pool was detected. The presence of two pools of DNA-bound lipids was also demonstrated in *S. typhimurium* [31]; these pools consist

of phospholipids (unidentified) and neutral lipids (di-, triglycerides, free fatty acids). The authors mentioned above [31] used to isolate DNA through the Marmur's method [35], and consequently, the lipid content is depended on the DNA molecular mass. It is to be stressed that the authors have determined for the first time the composition of fatty acids of two DNA-bound lipid pools and showed that the loosely bound pool is much richer in saturated fatty acids than the tightly bound pool. Thus, the DNA-bound lipid pools is a dynamic system, while in the malignant cell, the lipid pools are recoded.

4. The functions of the two pools of DNA-bound lipids

We believe that the presence of two pools of DNA-bound lipids is not accidental, and they fulfill important functions in nuclei. The following facts favor this suggestion. Removal of the loosely bound pool (with 35% ethanol, or with 1% Triton X-100 or with phospholipase C and A without hydrodynamic shifts) leads to the three-fold lowering of the DNA molecular mass (from 3×10^8 to 1×10^8 Da) [21], which presupposed the participation of the loosely bound pool in the replicon, loop-like organization of DNA. Besides, the extraction of this lipid pool activates RNA synthesis in the RNA-polymerase system.

The composition of tightly bound lipid pool is closed to the composition of the nuclear matrix [21], which assumes the participation of these lipids in fixation of DNA loops to the nuclear matrix, since the points of fixation of DNA onto the nuclear membrane of *Bacilus subtilis* are enriched with cardiolipin [40]. From our point of view, cardiolipin can play a special role in the function of lipid pools.

First of all, we will offer the arguments in favor of the structural–functional role of cardiolipin. Under this DNA isolation procedure [34], cardiolipin appears to be the major DNA-bound phospholipid of eukaryotic cells, bacteria, and phage [18–22]. In contrast to other phospholipids, all amount of chromatin's cardiolipin is localized in DNA, which could be explained by the availability of the common interphosphate motif between cardiolipin and DNA [41]. Cardiolipin also activates the protein responsible for initiation of *E. coli* replication (dnaA protein) [42,43] and SV-40 virus T-antigen [44], regulates activity of DNA polymerase α, β, γ [13,45], as well as activity of RNA polymerase [13,45,46] and DNA topoisomerases I and II [47,48]. Cardiolipin decondences chromatin histone H1 and removed from linker DNA in chromatin and nucleosomes [1,2]. Using the circular dichroism method, it was found that DNA B-form to A-form transition (active transcription) is elaborated by the presence of cardiolipin [21]. The content of DNA-bound cardiolipin is increased in S-phase of cell cycle (DNA synthesis), but lowered to normal level in G_2-phase [18,21]. It is calculated that in one rat liver cardiolipin molecule corresponds to two nucleosomes, while there is one cardiolipin molecule in one nucleosome (200 pairs of bases) in S-phase of cell cycle. From the findings above, it might be assumed that cardiolipin represents a phospholipid of proliferation [49].

In further discussion, we shall add arguments in support of the hypothesis that two pool of DNA-bound cardiolipin have different functions.

(1) The content of loosely bound cardiolipin varies in different eukaryotic cells and correlates with the content of euchromatin DNA (active transcription and replication); the higher the percentage of euchromatin DNA [50], the greater is the amount of loosely bound cardiolipin into DNA [21]. It is important that in the nonactive genome of pigeon erythrocytes, where there is no euchromatin DNA, the cardiolipin is only found in trace amounts (0.015 μg/mg DNA), while in T2 phage, this cardiolipin pool is lacking altogether [20,21].

(2) The content of cardiolipin in the pools depends on the phase of the cell cycle in the regenerating rat liver. The content of loosely bound cardiolipin was increased by three-fold in S-phase of cell cycle, while the content of tightly bound one was not changed at all. In G_2-phase, the content of loosely bound cardiolipin was decreased to normal value, and the content of tightly bound appeared to decrease four-fold than the normal value.

(3) The amount of cardiolipin in the two pools depends on DNA conformation: the amount of loosely bound cardiolipin of the supercoiled DNA is two-fold less than that of tightly bound one, and *vice versa*; and the relaxed DNA contains twice of the amount of loosely bound cardiolipin than the supercoiled DNA [21].

Further, it was shown [21] that partial removal of lipids (by 35% ethanol or 1% Ttriton X-100, or phospholipase C) from DNA preparations of rat thymus, liver, and loach sperm increased RNA synthesis *in vitro* in the presence of exogenous RNA polymerase from rat liver. In the process, an inverse correlation was observed: the greater the amount of lipids in the DNA, the weaker the transcription. This dependence is most clearly observed with loosely bound cardiolipin. Moreover, the T2 phage DNA in which the loosely bound lipid pool, particularly cardiolipin is absent at all, has the maximal transcription level (35,000 counts/min, inclusion of /^{3}H/-uridine). For comparison, rat thymus chromatin included 6000 counts/min, and supramolecular DNA complex (SC-DNA) from thymus—11,900 counts/min. It is necessary to note that DNA preparations contain endogenous RNA polymerase (see Table 4 in Ref. [21]), its activity in liver being twice higher than in thymus. It was shown that both the endo- and exoenzyme activities depend on the content of precisely loosely bound cardiolipin in DNA preparation compared to other lipids. Thus, there is an inverse correlation, which takes place between the endoenzymatic activity and the loosely bound cardiolipin in the DNA. Analogous dependence was revealed in experiments with X-rays irradiated rats at lethal dose of 10 Gy [21]. After 2 min of irradiation, the loosely bound cardiolipin content is decreased by 50%, while RNA polymerase is activated by

Table 4
Dependence of endogenic RNA polymerase activity on the content of loosely bound cardiolipin in rat thymus and liver supramolecular DNA complex (SC-DNA) before and after 2 min of γ-irradiation at lethal dose of 10 Gy (mean values from three to four experiments)

SC-DNA source	RNA synthesis (^{3}H-UTP counts/min)		Loosely bound cardiolipin (μg/10 mg DNA)	
	Control	Irradiation	Control	Irradiation
Thymus	5010±25	12,960±81	20.6±2.1	10.9±0.9
Liver	12,880±72	7626±35	12.9±1.1	19.2±1.7

Reaction mixture for enzyme consisted of: 25 mM Tris–HCl, pH 8.0; 150 mM KCl, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 1.5 mM dithiotreitol, 1 mM ATP, CTP, GTP и 0.1 mM [^{3}H] UTP (10 mCi/mmol), 10 mg DNA. After a 15-min incubation at 37 °C, cold 5% aq. trichloroacetic acid was added, precipitated at filter, and washed with ethanol and ether. Radioactivity of filters was measured using counter "Tritiotomatic" (Belgium).

two-fold and *vice versa*; the two-fold increase of loosely bound cardiolipin in the liver is accompanied by the two-fold decrease of the enzyme activity.

It was shown in our radiobiological studies [30] that the composition of two DNA-bound lipid pools in thymus and liver undergoes essential changes with time (2 min, 2 h, or 6 h) after γ-irradiation of rats with a lethal dose of 10 Gy. These changes influence, in various degrees, all individual fractions of neutral lipids and phospholipids, that is, the effect of redistribution between and within the pools takes place. This effect is most expressed, and always irreversible in the case of tightly bound cardiolipin and cholesterol into the radiosensitive thymus along with irreversible supramolecular DNA complex degradation. However, in the radioresistant liver by 6 h, two processes are developing simultaneously: restoration of supramolecular DNA complex and of initial cholesterol content. Thus, the DNA–lipid component wholly gets irreversibly destroyed after 6 h of irradiation in both the thymus and liver; but in the liver, the restoration of cholesterol correlates with restoration of DNA structure. It suggests that cholesterol plays an important structural role in the SOS preparation of supramolecular DNA level for maintaining basic metabolism in radioresistant liver cells. In favour of this fact, data show that 60% of chromatin's cholesterol is bound to the DNA [22]. Using the method of fluorescent probes, the possibility of the dual mechanism of cholesterol binding to DNA was demonstrated [51]: superficial binding at low cholesterol concentration (DNA/cholesterol=15:24 μg/ml) and intercalation into helix at high cholesterol doses (15:45 μg/ml, respectively). Since cholesterol participated in the signal transduction at the cellular level [52], the possibility cannot be excluded that it can be an element of the signal system at both the chromatin level and the DNA-bound lipids' level. Along with this finding, it is shown that autoliposomes containing cholesterol enhance the resistance of radiosensitive rat thymocytes to X-irradiation at the dose of 10 Gy [53]. The reason for that is the capability of cholesterol to protect cells against the radiation due to the suppression of free radical damage [54].

5. Conclusions

The natural specific DNA-bound lipids (neutral lipids and phospholipids) were isolated from different eukaryotic cells (normal and cancer), bacteria, and T2 phage. It was established from the critical analysis of literature data that the DNA preparations, isolated by various authors [17–21,23,31–33,38] using a variety of techniques [32,34,35] and from several sources, contain minor amount (1–3%) of the DNA-bound lipids. These lipids consist of two pools, differing in the degree of their binding to DNA (loosely bound and tightly bound lipids), in their compositions, and functions. The composition of two lipid pools changes depends on metabolic activity of genome, cell cycle, malignant transformation, and DNA conformation (supercoiled form–relaxed form DNA transition). Cardiolipin and cholesterol put in the most contribution in these effects. From our point of view, the DNA-bound lipid pools may be the elements of nuclear chromatin signal transduction that take part in the post-genome regulation of nuclear metabolism. Taking these facts into account, further studies of the composition and functions of human DNA-bound lipid pools as an integral part of genome are very important perspective for the regulation of gene expression and functional genomics.

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