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Nuclear lipid droplets: A novel nuclear domain

J.P. Layerenza [a], P. González [b], M.M. García de Bravo [a], M.P. Polo [a], M.S. Sisti [a], A. Ves-Losada [a,c,*]

[a] INIBIOLP (CCT-La Plata-CONICET-UNLP), La Plata, Buenos Aires, Argentina
[b] Cátedra de Patología, Facultad de Ciencias Médicas, UNLP & CIC-Pcia, Buenos Aires, Argentina
[c] Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, UNLP, La Plata, Buenos Aires, Argentina

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ABSTRACT

We investigated nuclear neutral-lipid (NL) composition and organization, as NL may represent an alternative source for providing fatty acids and cholesterol (C) to membranes, signaling paths, and transcription factors in the nucleus. We show here that nuclear NL were organized into nonpolar domains in the form of nuclear-lipid droplets (nLD). By fluorescent confocal microscopy, representative nLD were observed in situ within the nuclei of rat hepatocytes in vivo and HepG2 cells, maintained under standard conditions in culture, and within nuclei isolated from rat liver. nLD were resistant to Triton X-100 and became stained with Sudan Red, OsO4, and BODIPY493/503. nLD and control cytosolic-lipid droplets (cLD) were isolated from rat-liver nuclei and from homogenates, respectively, by sucrose-gradient sedimentation. Lipids were extracted, separated by thin-layer chromatography, and quantified. nLD were composed of 37% lipids and 63% proteins. The nLD lipid composition was as follows: 19% triacylglycerols (TAG), 39% cholesteryl esters, 27% C, and 15% polar lipids; whereas the cLD composition contained different proportions of these same lipid classes, in particular 91% TAG. The TAG fatty acids from both lipid droplets were enriched in oleic, linoleic, and palmitic acids. The TAG from the nLD corresponded to a small pool, whereas the TAG from the cLD constituted the main cellular pool (at about 100% yield from the total homogenate). In conclusion, nLD are a domain within the nucleus where NL are stored and organized and may be involved in nuclear lipid homeostasis.

1. Introduction

Cellular nuclei are an evolutionary development of the eukaryotic cell that enables a critical compartmentation of the processes of replication, transcription, pre-mRNA splicing, and ribosome assembly among other fundamental cellular functions.

Nuclear lipids play an active role in cell proliferation, differentiation, and apoptosis and are essential structural and functional components of the nucleus. The lipids, however, represent only 16% of the nuclear composition, with the remaining nuclear constituents consisting of proteins and nucleic acids [1,2].

In liver cells, the location of the nuclear lipids is not restricted to the nuclear membranes alone since the membrane-depleted nuclear matrix (Mx) constitutes a second lipid pool. The nuclear polar lipids (PL), particularly glycerophospholipids (GP)—though also found within the Mx—are, however mainly located in the nuclear membranes [3–5]. Moreover, Albi et al. determined that 10% of nuclear PL were associated with chromatin [4].

Up to now, since nuclear-lipid research has focused on PL, the information on neutral lipids (NL) is scarce. In 1970 Kleinig characterized triacylglycerols (TAG), cholesterol (C), and cholesteryl esters (CE) in the nuclei from pig liver [6], while Keenan et al. determined the composition of these same NL in nuclear membranes from bovine liver [7]. Later, TAG as well as CE and C were analyzed in nuclei from bovine and rat liver [8,9].

The aim of this work was to study nuclear-NL organization since those lipid species may represent alternative sources of nuclear fatty acids (FA) and signaling lipids to the nuclear membranes within locations for ready availability. Our hypothesis was that nuclear NL are organized within the nucleus in domains homologous to those of the cytosolic lipid droplets (cLD).

2. Materials and methods

2.1. Materials

Silica gel G precoated 20×20 cm thin layer chromatography (TLC) plates with or without a concentrating zone of 2.5×20 cm were from Merk (Buenos Aires, Argentina) and lipid standards from Nu-Chek Prep Inc. (Elysian, MN, USA). All chemicals and solvents were of analytical and HPLC grade, respectively. Rabbit polyclonal antibodies against ACAT-1 and Na^+/K^+−ATPase α, goat polyclonal antibodies against Lamin A, PLIN1 (perilipin) and SC-35; horseradish-peroxidase-conjugated (HRPc) rabbit anti(goat-IgG) antibody; and HRPc goat anti(mouse IgG) antibody

* Corresponding author at: INIBIOLP, Facultad de Ciencias Médicas, calles 60 y 120, 1900, La Plata, Argentina. Tel.: +54 221 482 4894; fax: +54 221 425 8988.
E-mail address: avlosada@biol.unlp.edu.ar (A. Ves-Losada).

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were purchased from Santa Cruz, Biotechnology, Inc. (Santa Cruz, CA, USA). Rabbit antibody against the voltage-dependent–anion-channel protein (VDAC) was obtained from Thermo Scientific (Rockford, IL, USA), monoclonal antibodies against LAP2β and p54[nrb] from BD Biosciences (San Jose, CA, USA), HRPc goat anti(rabbit IgG) antibodies from Thermo Scientific (Rockford, IL, USA), Alexafluor-594-labeled chicken anti(mouse IgG) from Invitrogen (Buenos Aires, Argentina), and Cy3-labeled monkey anti(goat IgG) from Jackson ImmunoResearch (West Grove, PA, USA). The TAG Color Kit™ was from Wiener Laboratories (Rosario, Santa Fe, Argentina); DAPI, Sudan Red, and osmium tetroxide (OsO_4) from Sigma-Aldrich (St. Louis, MO, USA); and the BODIPY 493/503 from Invitrogen (Buenos Aires, Argentina).

2.2. Dye preparation

Solutions of BODIPY 493/503 stock (1 mg/ml), Sudan Red (0.5 mg/ml), and osmium tetroxide 2.5% [w/v] were prepared by dissolution in absolute ethanol, 70% [v/v] ethanol, and buffer A (1.4 mM/l NaH_2PO_4, 8 mM Na_2HPO_4, and 150 mM NaCl; pH 7.40); respectively.

2.3. Animals

Experiments were performed on 180 to 200 g 60 to 70 day-old male Wistar rats. The rats were housed in rooms with 12:12 h light–dark diurnal cycle (midnight being the midpoint of the dark period), and the experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals (1996, National Academy Press). The animals were maintained on a commercial standard pellet diet (50% [w/w] Cargill mouse and rat chow, (Pilar, Buenos Aires, Argentina) and 50% [w/w] ACAI mouse and rat chow (San Nicolás, Buenos Aires, Argentina)) plus tap water *ad libitum*. The diet contained (by weight) 20% proteins and 4% total lipids with a FA composition of 15.6% 16:0, 1.1% 16:1, 6.3% 18:0, 26.9% 18:1 ($n-9$), 1.6% 18:1 ($n-7$), 42.7% 18:2 ($n-6$), 5.7% 18:3 ($n-3$), and trace amounts of 14:0, 20:4 ($n-6$), and 22:6 ($n-3$).

2.4. Preparation of rat-liver homogenates and nuclear fractions

Highly purified nuclei were isolated from liver homogenates by sucrose-gradient ultracentrifugation after the method of Blobel and Potter [10], as modified initially by Kasper [11], and then slightly further as follows: all rats were killed at 8 a.m. to equalize circadian-rhythm effects [12]. After decapitation, the animals were bled out freely and the excised livers first rinsed in cold buffer B (50 mM Tris–HCl, 2.5 mM KCl, 5 mM $MgCl_2$; pH 7.5) containing 0.25 M sucrose (buffer B-sucrose) and then immersed in ice before removal of connective tissue and weighing. The tissue was finely minced with scissors and washed two times with buffer B-sucrose. After addition of two volumes of this same solution per *g* liver and transfer to a 24-ml Potter–Elvehjem homogenizer equipped with a Teflon pestle, the tissue was homogenized at *ca.* 1,700 rpm. After about 15 up-and-down strokes to release the nuclei, the homogenate was filtered through four layers of cheesecloth. Of the collected filtrate, 8 ml were transferred to each of six 38.5-ml polyallomer tubes followed by 14 ml of 2.3 M sucrose in buffer B and the contents mixed by inversion. The homogenate was gradually underlayered with 3 ml of 2.3 M sucrose in buffer B. The nuclei were pelleted by centrifuging at 106,750×*g* and 4 °C for 1 h. The heavy brownish-red plaque at the top of the tube was loosened by rimming with a small spatula and removed. The supernatant was decanted off by a quick inversion of the tube and then allowed to drain thoroughly. The residual sucrose solution was removed by squirting distilled water against the wall of the inverted tube without contacting the white nuclear pellet. After wiping the inside wall of the tube with an absorbent tissue, this wash was repeated followed by a final wash with buffer B. The nuclear pellet was then resuspended in 3 ml of 1.0 M sucrose in buffer B by agitation with a plastic stirring rod and the resuspension transferred to a 15-ml Dounce tissue grinder with a glass pestle. After homogenization and transfer to a 15-ml Corex centrifuge tube, the nuclei were sedimented at 4,100×*g* and 4 °C for 10 min; the supernatant was discarded; and after resuspension in buffer B-sucrose, the nuclei were washed by a final centrifugation at 900×*g* and 4 °C for 10 min.

In order to obtain the Mx, a quantitative removal of the nuclear envelope was effected by disruption of the nuclear-envelope with a low concentration (0.08%) of the nonionic detergent Triton X-100 followed by separation of the Mx on a sucrose gradient after the method of Vann et al. [13], slightly modified as follows [3]: after resuspension of the above pellet in 0.25 M sucrose in buffer C (10 mM Tris–HCl, 2 mM $MgCl_2$; pH 7.5) to give a final concentration of 8 mg protein/ml, a 0.5-ml aliquot was mixed with 20 ml of ice-cold 0.29 M sucrose in buffer D (5 mM Tris–HCl, 5 mM $MgCl_2$, 1.5 mM KCl, 1 mM EGTA; pH 7.4; containing 0.08% [v/v] Triton X-100) and incubated on ice for 20 min. The Mx were sedimented at 165×*g* and 4 °C for 6 min, the supernatant removed, and the pellet resuspended in 5 ml of 0.25 M sucrose in buffer C. After the underlaying of a 10-ml cushion of 0.5 M sucrose in buffer C, the Mx was pelleted at 165×*g* and 4 °C for 6 min, and this sedimentation was repeated.

The isolated nuclei and Mx were finally resuspended in 25% (v/v) glycerol in buffer E (10 mM Tris–HCl, pH 7.9). The protein concentration of all fractions was determined by the Lowry method with crystalline bovine-serum albumin as a standard [14]. Storage of all fractions was at −70 °C.

2.5. Criteria of nuclear purity

Morphologic and biochemical criteria were used to assess the purity of the nuclei isolated from rat liver. Nuclei were screened for homogeneity and uniformity by light microscopy, and further examined by electron microscopy, as previously described [2]. The Mx, obtained from the isolated hepatic nuclei as described above, were analyzed by electron microscopy in order to verify that the detergent had removed the double nuclear membrane [2]. Antibodies against ACAT-1, VDAC, Na^+/K^+−ATPase α, and PLIN1 were used in Western-blot analyses as marker proteins for the endoplasmic reticulum [15], mitochondria [16], plasma membrane [17], and cytosol [18], respectively. Lamin A and LAP2β were likewise employed as marker proteins for the nuclear fraction [19,20].

2.6. Electrophoresis and immunoblotting

Samples of liver homogenate, isolated whole nuclei, and Mx were prepared for electrophoresis by first adjusting the protein concentration to 2 mg/ml with Laemmli sample buffer F (125 mM Tris, 20% glycerol, 4% [w/v] SDS, and 2% [v/v] 2-mercapthoethanol plus bromphenol blue; pH 6.8) and then boiling at 100 °C for 6 min. Samples (50 μg protein per well) were resolved by SDS-PAGE in 0.75-mm-thick 10% (w/v) uniform slab gels at 120 V for 100 min in buffer G (25 mM Tris base, 192 mM glycine, 3.5 mM SDS; pH 8.3). The separated proteins were transferred to PVDF membranes (BioRad, Hercules, CA, USA) in the transfer buffer H (25 mM Tris base, 192 mM glycine, 20% (v/v) methanol; pH 8.3). Nonspecific binding sites on the membranes were blocked by an overnight incubation with 5% (v/v) nonfat milk in buffer I (buffer A plus 0.1% [v/v] Tween-20, pH 7.4). The membrane strips were then incubated with the primary antibody diluted in 1% (v/v) nonfat milk in buffer I (1:200 anti-ACAT-1, 1:1,000 anti-VDAC, 1:1,000 anti(Na^+/K^+−ATPase α), 1:200 anti(PLIN1), 1:200 anti(Lamin A), and 1:2,000 anti-(LAP2β)) for 1 h at room temperature and under gentle shaking. Immunoblots were developed through the use of secondary HRP-conjugated antibodies. Membranes were washed 6 times for