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PROSPECTS

Gangliosides of the Nuclear Membrane: A Crucial Locus of Cytoprotective Modulation

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Abstract The original concept of gangliosides as localized components of the plasma membrane has broadened in recent years with recognition of their presence in various intracellular pools as well. The nuclear envelope (NE), consisting of two unique membranes, is one such structure shown to contain members of the gangliotetraose family and possibly other sialoglycolipids. GM1 situated in the inner membrane of the NE is tightly associated with a Na^+/Ca^{2+} exchanger whose activity it potentiates in the transfer of Ca^{2+} from nucleoplasm to the NE lumen. This is in contrast to Na^+/Ca^{2+} exchangers of the plasma membrane which bind GM1 less avidly or not at all. This is believed due to different isoforms of exchanger, and a difference in topology of the exchanger relative to GM1. Cultured neurons from mice genetically engineered to lack gangliotetraose gangliosides such as GM1 were highly vulnerable to Ca^{2+}-induced apoptosis. They were rescued to some extent by GM1 but more effectively by LIGA-20, a membrane-permeant derivative of GM1 that traverses the plasma membrane more effectively than GM1 and inserts into the NE. As further indication of Ca^{2+} dysregulation, the mutant mice were highly susceptible to kainite-induced seizures which were attenuated by LIGA-20. This correlated with the ability of LIGA-20 to cross the blood–brain barrier, enter brain cells, insert into the NE, and potentiate the nuclear exchanger. GM1 in the NE, in association with nuclear Na^+/Ca^{2+} exchanger, is thus seen as contributing to Ca^{2+} regulation within the nucleus and in the process exerting a cytoprotective role. J. Cell. Biochem. 97: 893–903, 2006. © 2006 Wiley-Liss, Inc.

Key words: gangliosides; nuclear membrane; nuclear calcium; sodium calcium exchanger; GM1-sodium calcium exchanger complex; cytoprotection by nuclear exchanger/GM1

Considering the large volume of information acquired over the years on the structure, metabolism, and function of gangliosides in the plasma membrane of numerous cell types, it is perhaps surprising that we are only at the beginning stage of inquiry concerning gangliosides of the nuclear membrane. This development may be related to the current revolution in the understanding of nuclear lipids in general. Although long recognized as nuclear components, lipids were initially viewed as limited in function to that of structural support for the nuclear envelope (NE). This likely reflected their relative paucity in other nuclear compartments and the limited knowledge of lipid signaling capabilities. This concept was dramatically revised in the last decade or two with growing awareness of lipid presence in the various domains of the nucleus interior and recognition of the powerful signaling, and modulating roles of lipids and their metabolic products in all nuclear compartments [for review: Tamiya-Koizumi, 2002; Irvine, 2003; Albi and Viola Magni, 2004; Ledeen and Wu, 2004].

NUCLEAR STRUCTURE

The nucleus is now viewed as a highly structured organelle enclosed within the two

Abbreviations used: CGN, cerebellar granule neurons; CNS, central nervous system; Ctx B, B subunit of cholera toxin; Ctx B-HRP, cholera toxin B subunit linked to horseradish peroxidase; INM, inner nuclear membrane; NCX, Na^+/Ca^{2+} exchanger; NE, nuclear envelope; ONM, outer nuclear membrane; SDS–PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.

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membranes of the NE with a variety of intranuclear domains (Fig. 1). These domains are defined as structural compartments that are dynamically variable in relation to metabolic function [Maraldi et al., 1998]. The outer membrane of the NE is directly continuous with the endoplasmic reticulum and shares certain properties with the latter, while the inner

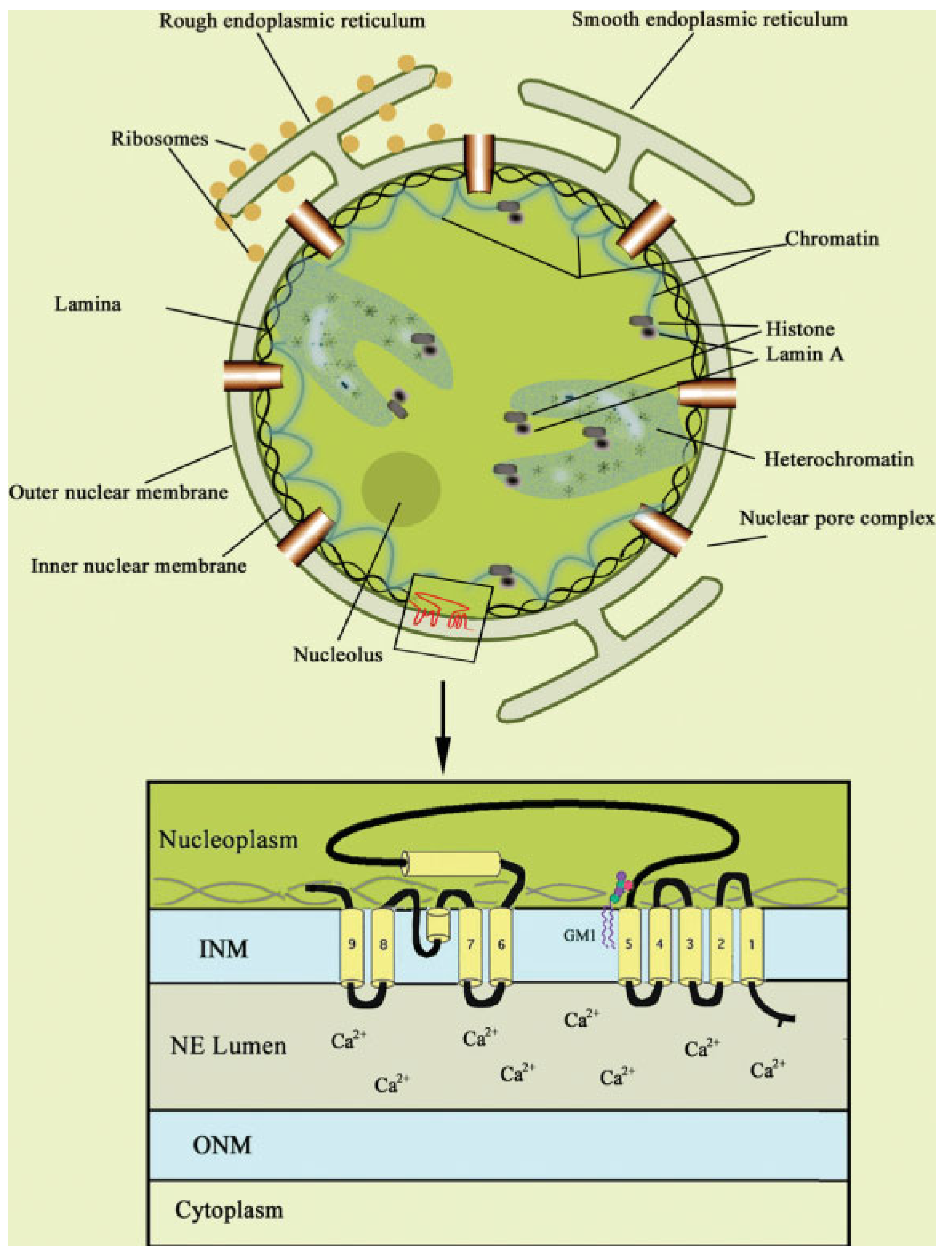

Fig. 1. Representation of nuclear structure with subnuclear domains, as presently conceived. The outer nuclear membrane (ONM) is continuous with the ER, while the inner nuclear membrane (INM) is closely associated with the nuclear lamina and has unique lipid composition. These two membranes are joined at the nuclear pore complexes that are distributed over the nuclear surface and permit passive flow of small molecules between cytoplasm and nucleoplasm. The lumenal space between the two membranes of the nuclear envelope (NE) is a storage site for Ca^{2+}. In addition to the NE, lipids have been shown to occur in intranuclear compartments such as nucleolus, chromatin, and heterochromatin. The lower panel shows the proposed topology of NCX and GM1 in the nuclear membrane. The large loop between transmembrane units 5 and 6 is seen as located in the nucleoplasm (low Ca^{2+} side) in close proximity to the oligosaccharide chain of GM1. Note that this is opposite to the orientation in the plasma membrane in which these structures occur on opposite sides of the membrane. We propose that a positively charged amino acid (e.g., Arg) in the alternatively spliced segment produced by the B exon interacts with the negative charge of GM1 *N*-acetylneuraminic acid to produce the tightly associated NCX/GM1 complex. Reproduced in part from Figure 1 of Ledeen and Wu [2004] with permission of *J. Lipid Res*.

membrane has distinctly different lipid and protein compositions, and is intimately associated with the nuclear lamina and chromatin. The nuclear lamina comprise a meshwork of intermediate filaments termed lamins that provide structural stability. The two membranes of the NE are joined at the nuclear pores by the pore membranes, which are associated with the nuclear pore complexes. The latter are distributed over the entire nuclear surface and consist of multi-protein assemblies of ~125,000 kDa size containing 30–50 different kinds of proteins for a total of ~1,000 polypeptides per complex. These allow passive transfer of small and middle-sized molecules (<50 kDa) between cytoplasm and nucleoplasm. Passage of larger molecules is energy driven and requires a nuclear localization signal.

GANGLIOSIDES ARE WIDELY DISTRIBUTED IN ANIMAL PHYLA

Gangliosides are now recognized as ubiquitous components of virtually all vertebrate cells [Yu and Saito, 1989; Hakomori, 2001] and some invertebrate tissues as well [Sugita, 1979; Smirnova and Kochetkov, 1980; Zvezdina et al., 1989; Saito et al., 2001]. They have not yet been detected in plants or microorganisms. Their functional roles encompass a variety of physiological processes, commensurate with the broad structural diversity that characterizes these sialic acid-containing glycosphingolipids. The subgroup of gangliosides belonging to the gangliotetraose family (Fig. 2) constitutes the predominant sialoglycoconjugate type in vertebrate neurons, plasma membrane being their primary locus [Ledeen, 1989; Ledeen and Wu, 1992]. GM1, the prototypic gangliotetraose ganglioside, along with various other members of this family, are also found in other neural components, such as glia, as well a great many extra-neural tissues. Adding to the complexity is the presence of sialoglycolipids of other ganglioside families, which often occur concurrently with the gangliotetraose structures. Although major attention has focused on plasma membrane, mounting evidence has documented the existence of intracellular pools of gangliosides which, while quantitatively minor, are believed to fulfill important signaling, transport, and regulatory functions [Sonnino et al., 1981; Ledeen et al., 1988; Gillard et al., 1993].

GANGLIOSIDES OF THE NUCLEUS

One of the earliest indications that gangliosides occur in the nucleus was a description of their presence in every subcellular fraction of bovine mammary gland and rat liver, the level in nuclei from the latter being comparable to that in total homogenate [Keenan et al., 1972]. A subsequent report showed ganglioside content of the nuclear membrane from rat liver to be close to 10% that of plasma membrane, the dominant species being GM1 and GM3 [Matyas and Morre, 1987]. Another study reported ganglioside occurrence in purified nuclei from bovine mammary gland [Katoh et al., 1993]. A frequent and legitimate question in regard to such studies has been the purity of the isolated nuclei, viz. whether this subcellular fraction was truly free of other ganglioside-bearing elements such as microsomes and plasma membrane. For that reason our initial study of nuclei employed cytochemistry in conjunction with biochemical analysis, applied to nuclei from neuroblastoma cell cultures that could be isolated in greater purity than nuclei from whole tissue. Because of their relatively low lipid content nuclei have a higher buoyant density than virtually all other subcellular fractions, which greatly facilitates isolation. This was verified in our study by the virtual absence of markers for plasma membrane, golgi apparatus, and endoplasmic reticulum in nuclei from Neuro2A cells [Wu et al., 1995a]. Our findings were that GM1 and GD1a account for the large majority of the observable gangliosides and that these are predominantly localized in the NE. GM1 at that site, although possibly less abundant than in neuronal plasma membranes, was clearly observable with cholera toxin B subunit linked to horseradish peroxidase (Ctx B-HRP) as cytochemical indicator; similar observations were made with primary neurons (Fig. 3). A developmental study of rat brain nuclei revealed the presence of those and some additional gangliosides in large (presumably neuronal) nuclei from mature brain, and relatively more GM3 and GD3 in such nuclei from developing brain; smaller nuclei (presumably glial) had significantly less total ganglioside [Saito and Sugiyama, 2002]. As mentioned, isolation of

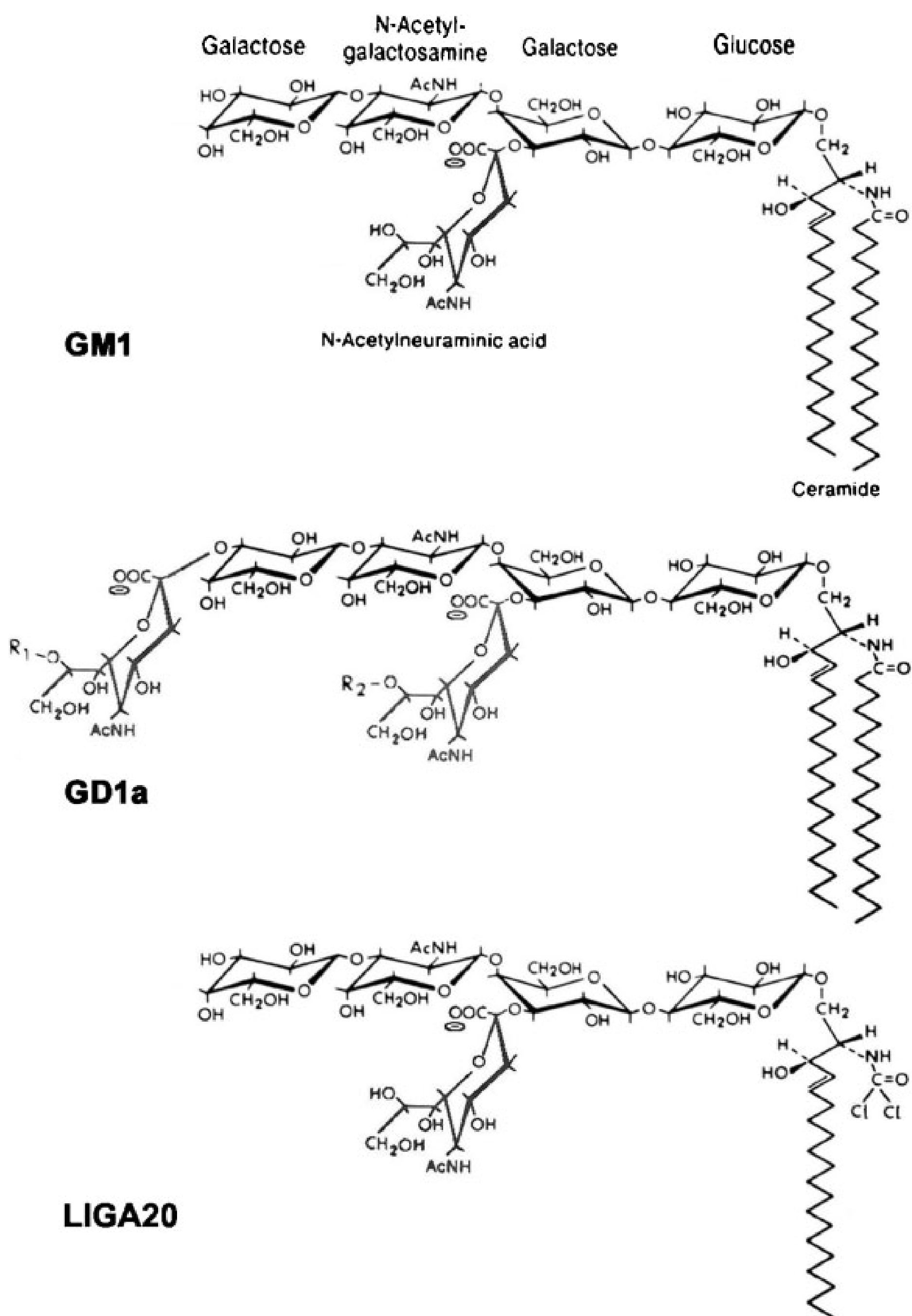

Fig. 2. Structures of GM1 and GD1a, the two major gangliotetraose gangliosides of the NE. Also shown is LIGA-20, a semisynthetic analog of GM1 in which the long chain fatty acid of the cermide unit is replaced by dichloroacetyl. This structural change renders it more membrane permeant than GM1. Additional gangliotetraose gangliosides occur in brain and other tissues in which R_1 and/or R_2 is (are) *N*-acetylneuraminic acid attached to GD1a. The negative charge(s) imparted by *N*-acetylneuraminic acid is (are) thought to facilitate interaction with positively charged peptides.

pure nuclei from whole tissue is more difficult than from cultured cells.

Efforts to more specifically localize GM1 utilized a procedure involving mild treatment with sodium citrate solution to selectively remove and isolate the outer membrane of the NE [Gilchrist and Pierce, 1993; Humbert et al., 1996]. The inner membrane was obtained from the resulting nucleus remnant, and shown to contain GM1 and GD1a [Xie et al., 2002]. Most

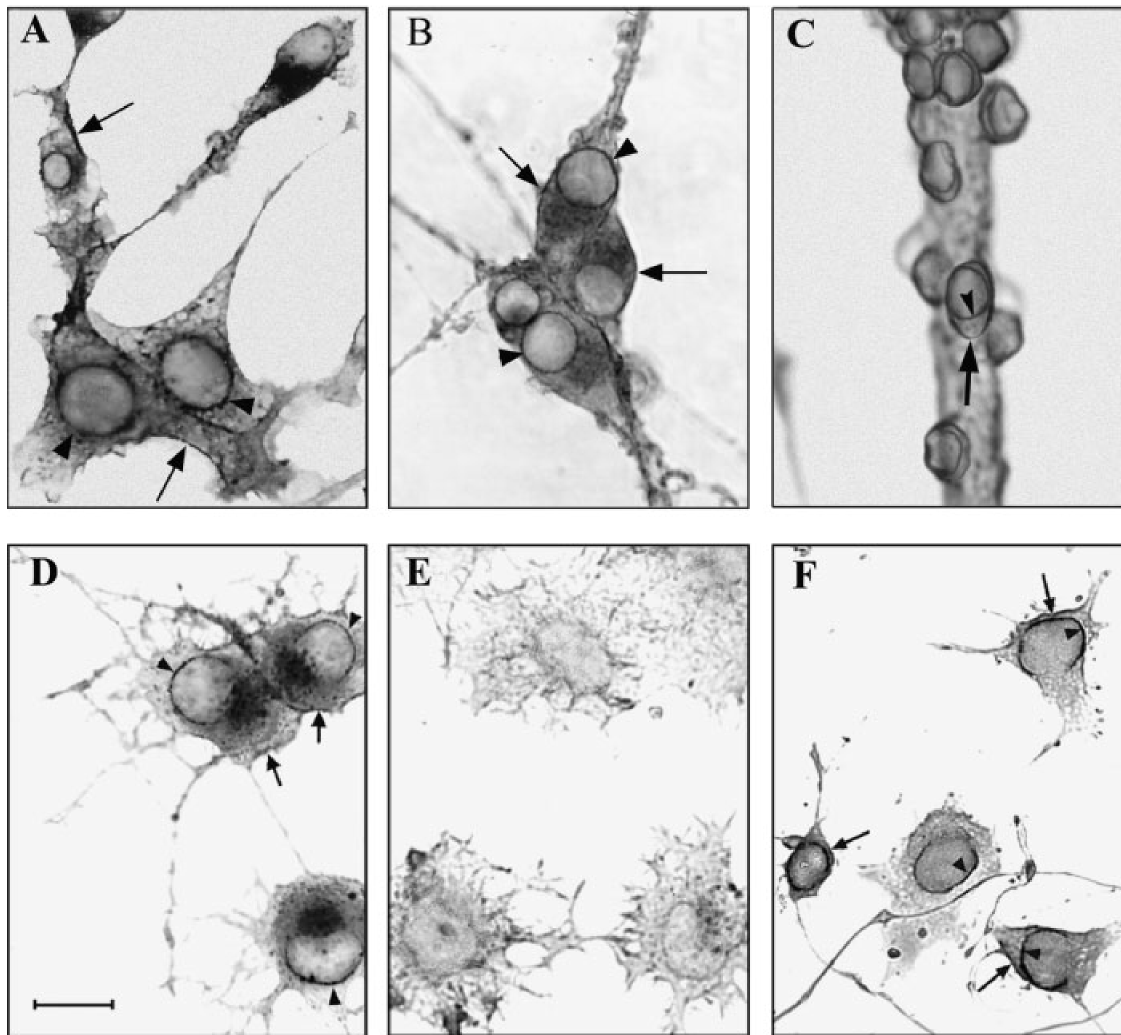

Fig. 3. Cytochemical detection of GM1 and Na^+/Ca^{2+} exchanger (NCX) in the NE of neural cell lines and primary neurons. Ctx B-HRP revealed GM1 in the NE of (**A**) differentiating Neuro2a cells, (**B**) cultured neurons from the superior cervical ganglion of embryonic rats, (**C**) cultured cerebellar granule neurons (CGN) from neonatal rat brain after 6 days in vitro, (**D**) differentiating NG108-15 cells, (**E**) undifferentiated NG108-15 cells. **F**: Anti-NCX antibody linked to HRP was used to detect NCX in the NE of differentiating NG108-15 cells. In all figures, arrowheads indicate visible staining of NE and arrows staining of plasma membrane. Note the paucity of GM1 staining in the NE of undifferentiated NG108-15 cells (E) as compared to the distinctive presence of GM1 in the NE of differentiating neuronal cells (A–D). Subpart A and C are reproduced from Figure 3 of Wu et al. [1995a] with permission of *J. Neuroscience*; Figure 3B is reproduced from Figure 4 of Kozireski-Chuback et al. [1999] with permission of Elsevier; subparts D–F are reproduced from Figure 1 of Xie et al. [2002] with permission of *J. Neurochem*.

surprising was the concurrent finding that GM1 at that locus occurred in very tight association with a Na^+/Ca^{2+} exchanger (NCX), a molecule not previously reported as a NE component (see below). Both GM1 and GD1a were also found in the outer nuclear membrane (ONM), likely subserving other functions. With respect to intranuclear domains, heterochromatin in nuclei from mouse epithelial cells was suggested to contain GM1 by virtue of binding both Ctx B and anti-GM1 antibodies [Parkinson et al., 1989]. Similarly, evidence for GD3 colocalization with nuclear chromatin was obtained immunocytochemically with rat cortical neurons subjected to β-amyloid peptide; this occurred just prior to the neurons entering S phase and apoptotic death [Copani et al., 2002].

FUNCTIONAL ROLE OF NUCLEAR GM1: POTENTIATION OF Na^+/Ca^{2+} EXCHANGER

Various studies demonstrating Ca^{2+} regulation to be among the demonstrated functions of plasma membrane GM1 [Wu and Ledeen, 1994;

Fang et al., 2002; Wu et al., 2004a] provided the rationale for seeking a similar role for nuclear GM1. Initial studies with cultured Neuro2a cells and their isolated nuclei provided some support for this [Wu et al., 1995b; Ledeen et al., 1998], but more definitive evidence came with demonstration of NCX isoforms in the NE that are strongly associated with and potentiated by GM1 [Xie et al., 2002]. High affinity association was demonstrated by immunoprecipitation with monoclonal anti-NCX antibody applied to NE isolated from NG108-15 cells followed by immunoblot analysis with polyclonal anti-NCX antibody and a parallel blot utilizing Ctx B-HRP (Fig. 4). These parallel blots showed GM1 to remain associated during sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS–PAGE) with NCX isoforms at 70, 160, and 220 kDa, but not the NCX at 120 kDa. These associations are rationalized in terms of NCX isoform variation and topological considerations (see below). Such tenacious association of a ganglioside with a protein it modulates is rare but not unprecedented [Mutoh et al., 1995; Misasi et al., 1998]. We have shown that the high affinity association of GM1 and NCX in the NE involves charge–charge interaction with requirement for a positively charged moiety in NCX [Xie et al., 2004a].

The immunoblot pattern of exchanger isoforms in the NE resembled that from plasma membrane in most respects (Fig. 4), but with the notable difference that none of the latter isoforms were associated with GM1 following SDS–PAGE. A looser association may occur but this remains to be tested. The GM1-associated band at 160 kDa, more prominent in the NE of differentiated cells, was reported to result from shift in gel position of the 120 kDa band due to conformational change [Santacruz-Toloza et al., 2000]; this is believed to represent the mature protein. The band complex at

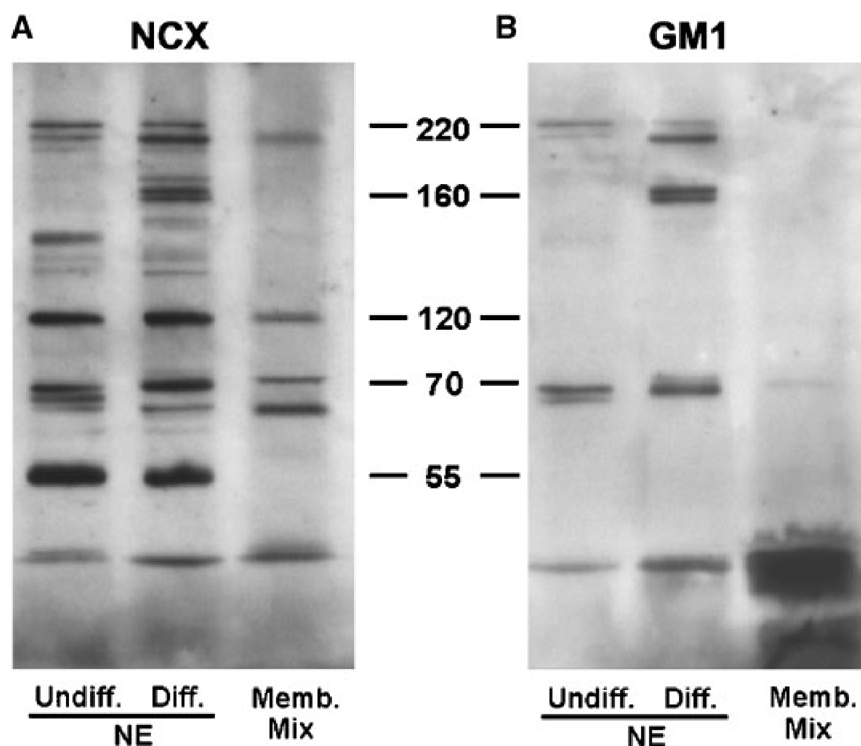

Fig. 4. Immunoprecipitation and immunoblot of the Na^+/Ca^{2+} exchanger associated with GM1 in the NE. The NE and mixture of non-nuclear cell membranes (Memb. Mix, including plasma membranes) were prepared from KCl-differentiated (Diff.) or undifferentiated (Undiff.) NG108-15 cells. Each was dispersed in 1% Triton X-100 and immunoprecipitated with monoclonal anti-NCX antibody plus protein L-agarose beads. The precipitated proteins were subjected to 7% non-reducing SDS–PAGE, transferred to PVDF membrane and blotted with polyclonal rabbit anti-NCX (**A**). A parallel SDS–PAGE gel was transferred to PVDF and blotted with Ctx B-HRP (**B**). NCX isoforms from the NE appearing at 220, 160, and 70 kDa remained associated with GM1 throughout SDS–PAGE and blotting. In contrast, NCX isoforms from the membrane mixture (containing plasma membrane) showed no retention of GM1 during SDS–PAGE, although the heavy staining at the migration front of Memb. Mix. (**lane 3** of B) suggested GM1 might have been loosely associated with one or more of these isoforms. Reproduced from Figure 3 of Xie et al. [2002] with permission of *J. Neurochem.*

$\sim$70 kDa is thought to represent a specific splicing product [Van Eylen et al., 2001] and/or proteolytic products, while that at $\sim$220 kDa could indicate a dimer (of 120 kDa) or trimer (of 70 kDa). Despite their parallel migration our results suggest the NCX components of the nuclear and plasma membranes represent different isoforms.

A physiological consequence of GM1 association with nuclear NCX is potentiation of Na^+/Ca^{2+} exchange activity, as seen in uptake experiments with isolated nuclei incubated in the presence of $^{45}Ca^{2+}$ [Xie et al., 2002]. In this system $^{45}Ca^{2+}$ was transferred from nucleoplasm to the NE lumen, consistent with NCX location at the inner membrane of the NE. Nuclei from differentiated NG108-15 cells, which contained elevated GM1 in the NE, affected significantly more $^{45}Ca^{2+}$ transfer than nuclei from undifferentiated cells which had little or no GM1 in the NE. When nuclei from undifferentiated cells were pre-incubated with GM1, Na^+/Ca^{2+} exchange activity was elevated. Moreover, the potentiating effect was specific for GM1 and was blocked by Ctx B. It is likely that Na^+/Ca^{2+} exchange in the NE is driven by a Na^+ gradient, as is the case for plasma membrane NCX. The in vitro assay employing isolated nuclei thus required elevation of Na^+ in the NE, accomplished by pre-incubating the nuclei in Na^+-containing medium in the presence of appropriate ionophores. Such loading is believed to occur naturally by a Na^+, K^+-ATPase in the inner membrane of the NE, reported to create high intra-lumenal Na^+ concentration [Garner, 2002]. Immunoprecipitation of NCX indicated GD1a is also associated with the exchanger, although this ganglioside did not potentiate exchange activity. It is possible that GD1a serves as a reserve of GM1, undergoing conversion to the latter as needed by a sialidase detected in the NE [Saito et al., 1996].

Placement of the NCX-GM1 complex at the inner membrane of the NE raises topological considerations both as to orientation with respect to GM1 and orientation of the complex relative to low and high Ca^{2+} concentrations. This type of exchanger in the plasma membrane mediates counter-transport of three Na^+ for one Ca^{2+}, while promoting uphill extrusion of cytosolic Ca^{2+} [Philipson and Nicoll, 2000]. One topological requirement for the so-called "forward mode" of exchange in the plasma membrane is that the large polypeptide loop between transmembrane segments five and six of NCX reside on the low Ca^{2+} (cytosolic) side of the membrane, and assuming the same requirement for NE, this loop would extend into the nucleoplasm to facilitate transfer of nucleoplasmic Ca^{2+} across the inner nuclear membrane (INM) to the high Ca^{2+} concentration pool within the NE lumen (Fig. 1). A key difference in GM1 orientation would result, the gangliotetraose oligosaccharide chain and NCX loop being on opposite sides of the plasma membrane but on the same side of the inner NE membrane. Support for the latter orientation of GM1 comes from the observed availability of GM1 oligosaccharide to Ctx B for cytochemical detection (Fig. 3) and blockage of Na^+/Ca^{2+} exchange [Xie et al., 2002]. In this orientation the negatively charged oligosaccharide of GM1 is able to interact with the large inner loop of NCX, some of whose isoforms are enriched in basic amino acids (see below).

The mammalian Na^+/Ca^{2+} exchanger forms a multigene family consisting of three subtypes, NCX1, NCX2, and NCX3, and various isoforms corresponding to splice variants have been identified for each [Thurneysen et al., 2002]. The NCX1 subtype, which predominates in many neural cells, contains six exons (A–F) that code for a portion of the large inner loop and give rise to the alternatively spliced isoforms of NCX1 [He et al., 1998]. Exons A and B are believed to be expressed in a mutually exclusive manner in combination with one or more of the remaining (cassette) exons [Kofuji et al., 1994]. Three exon B-containing isoforms were shown to be the predominant transcripts in rat cortical astrocytes and C6 cells [He et al., 1998], and that together with our finding that C6 cells contain only nuclear NCX [Xie et al., 2004b] suggests that isoforms containing the B exon are specifically expressed in the NE. Current studies in our laboratory appear to support this hypothesis in that Jurkat cells, which lack NCX, show NCX expression in the NE when transfected with plasmid containing the BCDEF isoform of NCX1 (work in preparation). The B exon was shown to contain four Arg residues [Quednau et al., 1997; Van Eylen et al., 2001], the positive charge of which could interact with the negative charge of GM1 to form the high affinity complex. This is in contrast to the A exon, hypothesized to be targeted to the plasma membrane, which contains only one Arg and might be less likely to associate tightly with GM1.

CELL TYPES POSSESING NCX AND THE NUCLEAR NCX/GM1 COMPLEX

There is growing interest in the variety of cell types that express one form or another of NCX in the plasma membrane, or in association with GM1 in the NE. These exchangers are most active in excitable cells such as neurons, cardiac myocytes, and secretory cells that experience rapid, several-fold elevation of $[Ca^{2+}]_i$ [Blaustein and Lederer, 1999; Philipson and Nicoll, 2000]. However, they also occur in cells of limited or no excitability. In agreement with others [He et al., 1998; Thurneysen et al., 2002], we found NCX to be expressed in astrocytes and C6 cells, but with a major difference: whereas astrocytes possess NCX in both plasma and nuclear membranes, our study showed that C6 cells express such activity only in the NE [Xie et al., 2004b]. The same study revealed that the Jurkat T cell line contains no NCX species in either membrane, in contrast to HeLa and NCTC cells that contain NCX in both membranes. As with neurons, NCX is tightly associated with GM1 in the NE of these non-excitable cells [Xie et al., 2004a]. Human lymphocytes, a mixed population of T and B cells, showed evidence of plasma membrane and nuclear NCX expression in some but not all cells. It remains to be determined whether this represents B cells, T cells, or a subpopulation of the latter. These investigations of cell type expression, while limited at present, suggest that the nuclear NCX/GM1 complex may be present in a greater variety of cells than those that possess NCX in the plasma membrane.

MODULATION OF NUCLEAR Ca^{2+} BY NCX/GM1

The NE resembles the plasma membrane in possessing a variety of mechanisms for controlling Ca^{2+} flux. Regulation of nuclear Ca^{2+} is of critical importance in relation to cell viability and signaling processes that govern virtually every aspect of cell behavior. The outer membrane of the NE is continuous with the ER and resembles the latter in containing SERCA-type Ca^{2+}-activated ATPase that pumps cytosolic Ca^{2+} into the ER (and hence NE) lumen [Gerasimenko et al., 1995]. An additional pathway for transfer of cytosolic Ca^{2+} into the NE is mediated by inositol 1,3,4,5-tetrakisphosphate, receptors for which were detected in the outer membrane [Koppler et al., 1993]. The NE lumen is continuous with the ER intermembrane space and thus provides a luminal storage site for a significant portion of nuclear Ca^{2+} [Petersen et al., 1998; Strubing and Clapham, 1999]. The INM contains a number of Ca^{2+}-release mechanisms regulated by Ins(1,4,5)P_3, cADP-ribose, and NAADP; activation of these results in Ca^{2+} transfer from NE to nucleoplasm [Stehno-Bittel et al., 1995; Humbert et al., 1996; Gerasimenko et al., 2003]. The apparent ability of Ca^{2+} to freely diffuse through the nuclear pore complexes under many (perhaps all) conditions has raised doubts concerning the possible existence of nuclear-cytosolic Ca^{2+} gradients (for review: Gerasimenko and Gerasimenko, 2004). However, some evidence has been cited for such gradients, such as the study showing that small changes in cytosolic Ca^{2+} caused equally rapid changes in nuclear Ca^{2+}, whereas larger cytosolic Ca^{2+} increases were attenuated in the nucleus [Al-Mohanna et al., 1994]. While the question of Ca^{2+} gradients remains controversial, there is growing evidence for independent regulation of Ca^{2+} within the nucleus [for review: Badminton et al., 1998]. Conceivably, the Na^+/Ca^{2+} exchanger at the INM, acting as a high capacity Ca^{2+} transporter to transfer nucleoplasmic Ca^{2+}, could contribute to such selective regulation.

CYTOPROTECTION AS FUNCTIONAL ROLE OF NUCLEAR NCX/GM1 COMPLEX

Sodium-calcium exchange activity potentiated by GM1 could serve a cytoprotective role in shielding the nucleus against prolonged elevation of cytosolic Ca^{2+}, a condition in which Ca^{2+} exit through nuclear pores would not be a protective option. Calcium is well known to have a critical role in apoptosis [Mattson and Chan, 2003], the nucleus being especially vulnerable. Cerebellar granule neurons (CGN) from mice engineered to lack GM2/GD2 synthase, with resultant deficit of GM2, GD2 and all gangliotetraose gangliosides were found deficient in calcium regulatory capability that resulted in apoptotic death when the cells were grown in the presence of high K^+ [Wu et al., 2001]. That this was due to absence of GM1 was suggested in the observation that CGN from these knockout mice could be rescued from elevated K^+ as well as excitotoxic levels of glutamate by GM1, and even more effectively by LIGA-20, a semisynthetic derivative of GM1 [Wu et al., 2004b]. This

correlated with the known efficacy of LIGA-20 (Fig. 2 for structure) in restoring Ca^{2+} homeostasis in normal CGN [Manev et al., 1990] and in CGN of the ganglioside-deficient cells, as determined by fura-2 ratiometric measurement of cytosolic Ca^{2+} [Wu et al., 2004b]. Involvement of the nucleus was indicated by in vivo studies using the above ganglioside-deficient mice which, when administered kainic acid, developed temporal lobe seizures of significantly greater severity/duration than was the case for normal mice [Wu et al., 2005]. The greater efficacy of LIGA-20 compared to GM1 in attenuating such seizes was correlated with the membrane permeant properties of the former and its ability to enter brain cells, insert into the NE and activate the subnormally active NCX of the NE. In that sense, it served as functional replacement for the missing GM1 in the mutants.

CONCLUSIONS AND SPECULATIONS

Our studies have established the presence of NCX isoform(s) in the NE of several cell types that is (are) tightly associated with GM1 and potentiated by this glycolipid. Situated at the inner membrane of the NE, the NCX/GM1 complex utilizes a Na^{+} gradient to transfer Ca^{2+} from nucleoplasm to the NE lumen, thereby contributing to nuclear Ca^{2+} homeostasis in a manner suggesting independent regulation by the nucleus. Studies in vivo and with cultured neurons indicate a cytoprotective function for this complex, as seen most strikingly in the greatly enhanced susceptibility to kainate-induced seizures in knockout mice lacking GM1 and in the rescue provided by LIGA-20, a membrane-permeant analog of GM1. Since such exchangers are bidirectional, they may under appropriate conditions release Ca^{2+} into the nucleoplasm as a signaling mechanism to modulate gene transcription and other Ca^{2+}-dependent processes. However, such signaling by NCX would parallel other Ca^{2+} release mechanisms of the INM and have yet to be demonstrated. It is of interest, in regard to the multiple cellular roles of GM1, that this ganglioside also influences Ca^{2+} regulation in the plasma membrane but, as mentioned, by an entirely different mechanism(s) [Fang et al., 2002; Ledeen and Wu, 2002; Wu et al., 2004a]. Additional functional roles may eventually be found for nuclear gangliosides, including those localized in the ONM.

In addition to understanding the physiological significance to cells possessing the nuclear NCX/GM1 complex, it is worth considering the functional consequences to cells lacking this cytoprotective mechanism. This pertains, for example, to the subpopulation of lymphocytes we observed with this deficiency in the NE, analogous to Jurkat T cells [Xie et al., 2004a]. Calcium signaling in T cells is recognized as highly complex, Ca^{2+} entry in such cells being long lasting and necessary for T cell function [Weiss et al., 1984; Lewis, 2001]. Attention is being directed to mechanisms by which immune effector cells disappear after eliminating foreign antigens and evidence was cited that such return of the immune system to rest is mainly due to programmed cell death of activated lymphocytes [Parijs and Abbas, 1998]. It may be speculated that death of this type could result from absence of the nuclear NCX/GM1 complex, such cells being vulnerable to Ca^{2+}-induced apoptosis resulting from the prolonged Ca^{2+} elevation characteristic of activated T cells. Absence of the nuclear NCX/GM1 complex might also be a factor in maintaining unresponsiveness or tolerance, to self-antigens. Further experimentation is needed to determine precisely which lymphocytes lack this nuclear mechanism and the manner in which this might relate to immune function.

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