

# Spreading the Wealth: Niemann-Pick Type C Proteins Bind and Transport Cholesterol

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Endocytosed cholesterol must be transferred from the environment (e.g., low-density lipoproteins) via the lysosomal system to the rest of the cell. In Niemann-Pick type C disease, this process fails. In a recent issue of *Cell*, Kwon et al. (2009) suggest how this transpires mechanistically by crystallizing a domain of a protein defective in this syndrome.

The failure to appropriately mobilize hydrophobic lipids in the mammalian lysosome can frequently be of devastating consequence. Patients suffering from one such defect, Niemann-Pick type C (NP-C) disease, generally succumb by their second decade of life, with a virtual absence of cerebellar function due to lysosomal accumulation of sphingolipids and LDL-derived cholesterol (Figure 1). Mutations in two genes, NPC1 (encoding a lysosomal transmembrane protein; Carstea et al., 1997) and NPC2 (encoding a lysosomal lumen protein; Naureckiene et al., 2000), independently confer 95% and 5% of NP-C disease cases, respectively. As is too often the case, the isolation of the disease genes has not provided a treatment or, until recently, even a mechanism by which low-density lipoprotein (LDL)-derived cholesterol traverses the lysosomal/endosomal network.

In a tour de force of papers, culminating in the current work (Kwon et al., 2009), Infante, Goldstein, Brown, and Kwon have characterized cholesterol binding by the NPC proteins and thereby conceptualize a model for the movement of LDL-derived cholesterol within and perhaps beyond the lysosome. In so doing, they have firmly established the NP-C disease pathway as a functional component of the LDL-receptor network of endocytosed cholesterol utilization. These studies emanated from the surprising observation that sterol binding by NPC1 is accomplished by an NH<sub>2</sub>-terminal “soluble” domain of the protein, unique to this gene family (Infante et al., 2008). In the current study, the crystal structures of

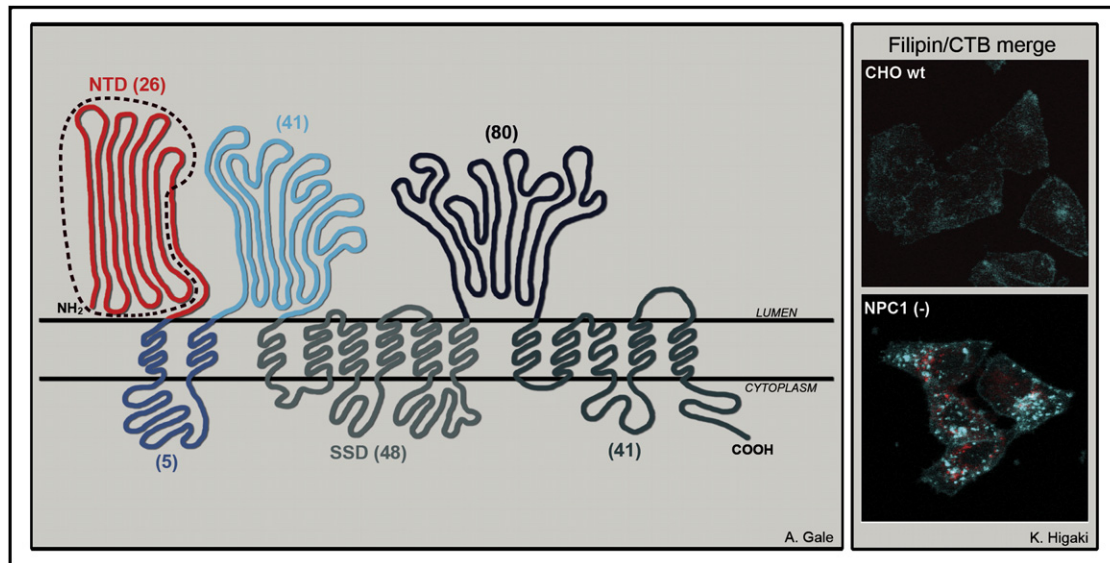
both apo- and ligand-associated forms of this domain (NPC1(NTD), residues 23–247; Figure 1) are defined. The crux of the proposed model rests upon the manner in which cholesterol is orientated in NPC1(NTD) and NPC2 as mirror images of each other. This implicates a head-to-tail donation of cholesterol between these proteins, followed by its transfer into the lysosomal membrane.

Kwon and colleagues also performed saturation mutagenesis of NPC1(NTD) and identified distinct subdomains within NPC1(NTD) that bind cholesterol from NPC2 or transfer cholesterol to liposomes in vitro. Such mutants failed to complement lipid egress in *npc1*<sup>-/-</sup> mutant cells, thus confirming the physiological relevance of NPC1(NTD) (Kwon et al., 2009). Amino acid identity in human, chimp, dog, cow, mouse, and yeast at 6 of 14 residues in NPC1(NTD) critical to cholesterol binding suggests a primordial need for binding sterols in the lysosome. Accordingly, the functions of orthologs separated by more than 2 billion years of evolution (e.g., yeast Ncr1p and human NPC1; Malathi et al., 2004) are apparently indistinguishable. Surprisingly, only 6 of the 14 critical cholesterol-binding sites are identical between human NPC1(NTD) and its paralog, NPC1L1. In contrast to binding cholesterol, transfer is a less-conserved process, as only 2 out of 11 of these sites are conserved from yeast Ncr1p to human NPC1 and NPC1L1.

The studies by Infante and colleagues were made with a relatively modest portion (17%) of the NPC1 molecule,

thus begging the questions: What is the rest of the molecule for, and are there other domains that bind sterols or other lipids? The other regions of NPC1 must have critical functions, given that NPC1(NTD) only contains 20 of the 241 disease mutations in NPC1 (Figure 1; inventoried in NPC-db <http://npc.fzk.de>). Prior to characterization of NPC1(NTD), the favored culprit for binding or responding to cholesterol was the sterol-sensing domain (SSD; residues 615–797; Figure 1). In common with similar domains in other proteins, the NPC1(SSD) has the potential to modulate protein-protein interactions in response to sterol levels. The Dallas model for handoff of cholesterol from NPC2 to NPC1 provides the first kinetic rationale to a longstanding hypothesis that there is a physical interaction between NPC2 and NPC1. Surprisingly, such interactions have been elusive and are presumably transient. To date, the only reported interaction of NPC1 is a cholesterol-mediated physical association with SKD1 (Ohsaki et al., 2006), a protein implicated in endosome-to-lysosome transport.

Under standard conditions, cholesterol crosses membranes by simple equilibration. This passive process is often catalyzed by energy-consuming membrane transporters, such as ATP-binding cassette proteins (e.g., ABCA1 and ABCG1). These chemical and genetic processes must be hindered in NP-C disease. For example, it is possible that the glycocalyx at the inner surface of the limiting membrane of the lysosome (as proposed by Kolter and Sandhoff, 2005) or the



**Figure 1. Schematic Representation of the NPC1 Protein and the Biochemical Hallmark of Niemann-Pick Type C Disease**

This structure (redrawn from Davies and Ioannou, 2000) emphasizes the domain-like nature of NPC1 and distinguishes the NPC1(NTD) characterized by Kwon et al. (2009) with a dashed line. Speculative transmembrane and soluble domains are indicated by the different colors. The sterol-sensing domain (SSD) has been characterized by mutagenesis and conservation with other related proteins. Chinese hamster ovary cells stained for cholesterol (filipin) and sphingolipids (cholera toxin B, CTB) show increased intracellular accumulation of these metabolites in NPC1 mutant cells. Accumulation of these lipids is the result of any one of 241 mutations distributed across the NPC1 protein. The number of mutations in each structural “domain” is indicated in parentheses adjacent to the respective domain. Images are courtesy of Ann Gale and Katsumi Higaki.

LBPA-enriched membranes of the multivesicular bodies (Kobayashi et al., 1999) may impede the free trafficking of cholesterol out of this organelle. Thus, even in the case of “normal” cholesterol transport from NPC2 to NPC1, an additional barrier must still be negotiated. Cyclodextrin, a cholesterol-binding agent, re-establishes normal and effective sterol egress even when the NPC1 and NPC2 proteins are dysfunctional (Liu et al., 2009). Perhaps the beneficial effects of cyclodextrin act at the glycocalyx or LBPA barrier? It is further possible that an as yet unidentified NP-C disease candidate (proposed by the authors of the current study) may activate the membrane-traversing forces, be enhanced by cyclodextrin, and/or represent the molecular sites at which NP-C phenotypes can be induced with progesterone and hydrophobic amines.

An ongoing debate is whether cholesterol is the primary ligand for NPC1 and whether sphingolipids are mere by-

standers that secondarily accumulate. If sphingolipids are not ligands for NPC1(NTD) or any other part of NPC1, then the argument for primary accumulation of cholesterol due to defective NPC1 would be favored. Similarly, do the N-terminal domains of family members, such as yeast Ncr1p or human NPC1L1, display similar ligand binding as a consequence of sequence conservation? In essence, the cumulative research by the group at Dallas has finally validated the longstanding prediction that NPC1 binds and transports sterols. In so doing, the field has at last advanced to a mechanistic phase.

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