Commentary

Niemann-Pick Type C Disease and Alzheimer's Disease

The APP-Endosome Connection Fattens Up

Ralph A. Nixon

From the Departments of Psychiatry and Cell Biology, New York University School of Medicine, Center for Dementia Research, Nathan Kline Institute, Orangeburg, New York

Niemann-Pick Type C (NPC) is an inherited neurodegenerative disease of childhood and adolescence that develops from a failure of cholesterol trafficking within the endosomal-lysosomal pathway. Although NPC differs in major respects from Alzheimer's disease (AD), intriguing parallels exist in the cellular pathology of these two diseases, including neurofibrillary tangle formation, prominent lysosome system dysfunction, and influences of apolipoprotein E $\epsilon 4$ genotype. Added to these similarities are new findings that some neuronal populations develop abnormalities of endosomes resembling those seen at the earliest stages of AD and also accumulate β -cleaved amyloid precursor protein (APP) and $A\beta$ peptides within endosomes. In this commentary, the common features of endosome dysfunction are reviewed. Emerging evidence that endosome dysfunction may lead to β -amyloidogenic APP processing or neurodegeneration by several different means is discussed. (*Am J Pathol* 2004, 164:757-761)

Niemann-Pick type C disease (NPC), an inherited lysosomal storage disorder, has long intrigued investigators of Alzheimer's disease (AD) as one of a very few disorders in which neurofibrillary tangles (NFT) robustly form in the brain in the absence of tau mutations or β -amyloid deposition.^{1,2} That the primary deficit in NPC involves dysfunction of cholesterol trafficking adds to the intrigue, given the mounting evidence that high cholesterol is a risk factor in AD³ and that the $\epsilon 4$ isoform of apolipoprotein E (ApoE), a protein carrier for cholesterol, promotes disease development in both disorders. With additional reports now linking changes in cholesterol homeostasis to altered APP processing and $A\beta$ generation,⁴⁻¹¹ the

pathobiology of NPC has become a potentially rich lode from which to mine clues about AD pathogenesis.

The studies reported by Jin et al¹² in this issue of *The American Journal of Pathology* tie the cellular mechanisms operating in NPC and early stage AD even more tightly by showing that amyloidogenic processing of APP in NPC neurons localizes to early endosomes, a compartment of the endocytic pathway that develops the earliest pathological changes yet known in AD^{13,14} and is implicated in amyloidogenesis in sporadic forms of AD and in Down's syndrome (DS).^{14,15} The striking parallels between the endosomal alterations in both diseases and the high disease-specificity of these alterations invite a deeper analysis into how a primary endosomal-lysosomal disorder can inform us about the origins of Alzheimer's disease, even when clinical and pathological manifestations of the two diseases differ.

The Niemann-Pick syndrome arises from inherited defects that cause either a cellular accumulation of cholesterol, as in the type C form of Niemann-Pick disease, or of sphingomyelin, in the case of Niemann-Pick types A and B. Depending in part on the age of onset, NPC can present initially as a systemic disease, featuring prominent hepatosplenomegaly, or as a neurological disease, characterized by cerebellar ataxia, bulbar dysfunction, and variable degrees of cognitive decline.^{16,17} Both systemic and neurological features ultimately coexist although visceral symptoms predominate in perinatal and infantile forms of NPC, while motor deficits and cognitive and psychiatric dysfunction predominate in the more common late infantile/juvenile forms and the rare adult cases. Death in the juvenile form, which is attributed

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Address reprint requests to Ralph A. Nixon, M.D., Ph.D., Department of Psychiatry, New York University School of Medicine, Center for Dementia Research, Nathan Kline Institute, 140 Old Orangeburg Road, Orangeburg, NY 10962. E-mail: Nixon@nki.rfmh.org.

largely to the progressive neurodegeneration, often occurs in the teens or twenties.

NPC neuropathology at the anatomical level differs considerably from that of AD. The most vulnerable neurons are Purkinje cells, accounting for the prominent ataxia seen clinically. Neurodegeneration, however, is progressive and widespread within cortical and subcortical neuronal populations. A "dying back" or retrograde pattern of degeneration is suggested by the striking degeneration of large fiber tracts in the human disease and in animal models of NPC.^{18–20} In the juvenile form of NPC, NFTs develop in significant numbers in the third decade¹ and contain PHF-tau, which is structurally and immunologically similar to that in AD tangles.^{21–23} Mouse, dog, and cat models of NPC exist, all arising from spontaneous mutations, and these models reproduce the main neurological and pathological features of the human disease.^{16,24,25}

The failure of cholesterol trafficking in NPC can be understood in part from its genetic basis, which involves mutations of either of two functionally related genes, NPC1 and NPC2, accounting respectively for 95% and 5% of the cases.^{26,27} The NPC1 protein, normally located primarily in lysosomes²⁸ contains a putative sterol-sensing domain common to other proteins involved in cholesterol homeostasis.²⁹ NPC1 influences the trafficking of NPC2, a protein that resides in the *trans*-Golgi network and late endosomes and is regulated by the cation-independent mannose-6-phosphate receptor (MPR215). Cells lacking MPR215, like cells in NPC, accumulate cholesterol in late endosomes³⁰ suggesting that MPR215 binding to NPC2 is important for endocytic cholesterol transport.

Cells with dysfunctional NPC1 or NPC2 accumulate unesterified cholesterol in late endosomes, which reflects a failure of cholesterol to efficiently exit this compartment and travel to the plasma membrane and ER. NPC cells can internalize LDL cholesterol, transport it to endosomes, and hydrolyze the LDL moiety, but once it is delivered to NPC1-containing late endosomes, a "lipid traffic jam" develops involving cholesterol, some other lipids and gangliosides, and MPR215.^{18,28,31,32} The traffic jam also traps endogenously synthesized cholesterol,³³ and impedes its delivery into distal axons, where it is required for membrane maintenance. Cholesterol in the brain, unlike other tissues, originates mainly from endogenous synthesis.³⁴ This transport failure in NPC, therefore, likely accounts for the special vulnerability of axons in the disease and their lowered potential for regeneration, as well as the impairment of ApoE-mediated cholesterol scavenging ability after axon injury.³⁵

Jin and colleagues used both primary cortical neurons and NPC brain tissue to assess the consequences of this lipid traffic jam on the metabolism of APP, which is known to be actively processed in the endocytic pathway.^{36–40} Exploiting an observation that U18666A, a class-II amphiphile, directly inhibits NPC1 function and induces an NPC-like phenotype in cells,⁴¹ they tracked cholesterol accumulation and β - and γ -cleaved products of APP within different endocytic compartments identified with antibody markers. As expected, cholesterol accumulated

in late endosome/lysosome compartments of U18666A-treated cells, as seen in NPC. Also consistent with earlier reports,^{9,10} A β 42 and the β -cleaved C-terminal fragment of APP, C99, accumulated in the cells, including a significant proportion of formic acid-insoluble and -soluble aggregated forms of these peptides. The absence of a U18666A effect on C99 and A β levels in a cell line overexpressing C99 suggested that the effects seen in the U18666A treated neurons were due at least in part to the increased β -cleavage of APP. A surprising finding revealed by immunocytochemistry, however, was that the antibodies recognizing A β and C99/APP-CTF did not decorate mainly the late endosomes/lysosomes that accumulated cholesterol but, instead, labeled rab 5-positive early endosomal compartments. The result was even more clear-cut in the Purkinje cells of a small set of human NPC cases where increased APP fragment immunoreactivities, corresponding presumably to the C99 elevations by Western blot analyses, co-localized with rab 5-positive early endosomes rather than the late endosomes.

Early endosomes were also abnormal in other ways. They were substantially enlarged and contained high levels of the lysosomal hydrolase cathepsin D suggesting that cathepsins were being partially rerouted to early endosomes in NPC Purkinje cells. Hippocampus and cerebral cortex, which are affected late in the course of NPC, did not display these abnormalities although intraneuronal A β 42 was detected in late endosomes of neurons in the CA1 area and entorhinal cortex of older adult subjects. These results on endosomal alterations in NPC are supported by findings in the mouse model of NPC¹¹ where brain levels of β CTF, A β 42, and A β 40 are also increased and presenilin is redistributed from the ER to early endosomes. Early endosomes in NPC1-disrupted CHO mutant cells and NPC1-deficient mouse brain also acquire more β CTF, A β 40, and A β 42, although A β accumulates in cholesterol-containing late endosomes in U18666A-treated CHO cells, suggesting that differences in cell type or the nature of the experimental cholesterol perturbation, including the source of cholesterol, may influence the details of the trafficking dysfunction.^{42,43}

The convergence on endosomes of many factors that promote amyloidogenesis is another remarkable example, in addition to neurofibrillary neurodegeneration, of the overlap between cellular mechanisms operating in NPC and AD. In Alzheimer's disease, alterations of rab 5-positive endosomes are the earliest appearing disease-specific cellular pathology. Rab 5-positive endosomes are enlarged in some neurons even before birth in Down syndrome, and this abnormality is evident in many neurons decades before the onset of dementia in these individuals.¹⁴ In sporadic Alzheimer's disease, early endosomes are already abnormal in pyramidal neurons of the neocortex at a stage of disease when Alzheimer-like (plaque and tangle) pathology is limited only to the hippocampus and entorhinal cortex. The endosomal changes in the neocortex at this very early stage of the disease coincide with the appearance of A β 40 and A β 42 in early endosomes and the initial rise in soluble A β levels.¹⁵ Rab 5-positive compartments are abnormally

enlarged, proteins facilitating rab 5 function such as EEA1 and rabaptin 5 are mobilized to early endosomes, and the expression of rab 4, an index of endosome recycling, is increased. Together these findings suggest that endocytosis is up-regulated and possibly dysfunctional. A similar endosomal phenotype is produced in cell lines overexpressing rab 5. As in NPC cells, β CTF is overproduced and appears in early endosomes of the rab-5-transfected cells, which also generate substantially higher amounts of $A\beta$ 40 and $A\beta$ 42 than control cells.³⁸ The enlarged early endosomes in Alzheimer's disease, as in NPC, also contain abnormally high levels of lysosomal hydrolases.¹³ This is at least partly due to a rise in MPR46 expression in the brain at the incipient stages of AD.⁴⁴ Overexpression of MPR46, or modified MPR constructs that deliver lysosomal hydrolases to endosomes substantially increases $A\beta$ 40 and $A\beta$ 42 production in cell lines.⁴⁴ Thus, two abnormal features of endosomes seen in AD and NPC, endosome enlargement and elevated hydrolase content, are tied mechanistically in AD brain to other known biochemical abnormalities that promote amyloidogenic cleavage of APP within the endocytic pathway. It will be interesting in the future to examine how MPRs promote mistrafficking of cathepsins and other proteins in NPC, especially in view of the mannose content of NPC2 and the importance of MPR215 in NPC2 function and cholesterol trafficking.³⁰

Endosomal pathology also develops in a genetic model of Down syndrome, the segmental trisomy 16 mouse (Ts65Dn), within the basal forebrain, hippocampus, and neocortex, which later exhibit aging-related atrophy and degenerative changes.⁴⁵ Triplication of *App*, one of the genes in the trisomic segment of chromosome 16, is required for endocytic abnormalities to develop,¹⁵ highlighting the importance of this AD-related gene in the development of this endosomal phenotype. The degenerative changes in these mice develop in the absence of β -amyloid deposition or neurofibrillary tangles, although soluble $A\beta$ peptide is overproduced in amounts disproportionately higher than the 50% increase expected based on *App* gene dosage. Recent immunocytochemical studies place this $A\beta$ mainly within rab 5-positive endosomes (Anne Cataldo, personal communication).

β -CTF and soluble, intracellular forms of $A\beta$ are increasingly being investigated as *App*-related moieties that may be the most critical to Alzheimer's disease pathogenesis.^{46–53} Soluble $A\beta$ levels correlate better than insoluble $A\beta$ with synaptic changes and neurodegeneration in AD,⁴⁷ and lowering soluble $A\beta$ levels improves behavioral deficits without reducing amyloid burden in some transgenic models.^{50,54} The recent findings in NPC and DS, therefore, raise interesting questions about the cause and effect relationship between endosomal dysfunction and intracellular β CTF and $A\beta$ and how each contributes to disease progression. In NPC, primary genetic defects of endosomal proteins clearly precede and cause β CTF and $A\beta$ overproduction, but whether these APP mutations play a role in the further neurofibrillary tangle formation and neurodegeneration is an interesting question. These same endosomal protein defects in NPC, however, also have more global effects

on endosomal/lysosomal function and trafficking including impaired transport of cholesterol and other constituents to distal axons.⁴³ These effects could easily account for neurodegeneration, based on growing evidence linking primary endosomal-lysosomal dysfunction to selective neurodegeneration.^{55–60}

In the Ts65Dn mouse model of Down's syndrome, endosomal abnormalities and intracellular $A\beta$ accumulation develop in some neuronal populations that are affected in AD and that, in these mice, later develop neurodegenerative changes. Intracellular or extracellular $A\beta$ accumulation, by itself, does not seem to cause this endosomal pathology,^{14,15} indicating that, like in NPC, endocytic pathway dysfunction likely precedes and leads to $A\beta$ overproduction.^{37,38,44} Evidence also supports the possibility that, as in NPC, endocytic dysfunction in AD and DS could contribute to neurodegeneration through mechanisms that are $A\beta$ -independent.^{61–64} For example, key signaling functions of early endosomes are defective in Ts65Dn mice, leading to impaired neurotrophic stimulation of neurons.⁵⁹ Growth factor signaling in normal neurons involves receptor-mediated endocytosis and a subsequent cascade of signaling events involving proteins associated with the early endosome, collectively comprising the "signaling endosome."^{65,66} Successful trophic action likely requires the signaling endosome to be transported back from nerve terminals to the cell body.⁶⁶ In the Ts65Dn mouse, retrograde NGF signaling is defective^{59,66} in basal forebrain cholinergic neurons, which seems to be responsible for the age-related neurodegenerative changes in these neurons. Whether or not this signaling failure involves impaired transport of the signaling endosome in these neurons is unknown. But if it turns out to be the case, it is tempting to speculate that such a defect might be linked to *App*, which is critical to development of the abnormal endosomal phenotype in Ts65Dn mice. Indeed, APP undergoes anterograde and retrograde axonal transport^{67,68} and is believed to act as a receptor for the axonal transport of kinesin-related vesicular cargo.⁶⁹ It can be imagined, therefore, that modifying the kinesin-interacting cytoplasmic tail of APP by phosphorylation or proteolysis, including elimination of the tail by γ cleavage, could greatly influence trafficking. It would be interesting to know, in this context, how cholesterol may influence APP's role in endosomal transport. Whether or not all of these events turn out to be related, investigations of NPC will undoubtedly reveal additional insights into the close relationship between dysfunction of the endosomal-lysosomal system and neurodegenerative disease.

References

1. Love S, Bridges LR, Case CP: Neurofibrillary tangles in Niemann-Pick disease type C. *Brain* 1995, 118:119–129
2. Ohm TG, Treiber-Held S, Distl R, Glockner F, Schonheit B, Tamanai M, Meske V: Cholesterol and tau protein: findings in Alzheimer's and Niemann Pick C's disease. *Pharmacopsychiatry* 2003, 36(Suppl 2): S120–S126
3. Poirier J: Apolipoprotein E and cholesterol metabolism in the patho-

- genesis and treatment of Alzheimer's disease. *Trends Mol Med* 2003, 9:94–101
4. Bodovitz S, Klein WL: Cholesterol modulates α -secretase cleavage of amyloid precursor protein. *J Biol Chem* 1996, 271:4436–4440
5. Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K: Cholesterol depletion inhibits the generation of β -amyloid in hippocampal neurons. *Proc Natl Acad Sci USA* 1998, 95:6460–6464
6. Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, Runz H, Kuhl S, Bertsch T, von Bergmann K, Hennerici M, Beyreuther K, Hartmann T: Simvastatin strongly reduces levels of Alzheimer's disease β -amyloid peptides A β 42 and A β 40 in vitro and in vivo. *Proc Natl Acad Sci USA* 2001, 98:5856–5861
7. Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurti K, Duff K, Pappolla MA: Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis* 2000, 7:321–331
8. Launer LJ, White LR, Petrovitch T, Ross GW, Curb JD: Cholesterol and neuropathologic markers of AD: a population-based autopsy study. *Neurology* 2001, 57:1447–1452
9. Yamazaki T, Chang TY, Haass C, Ihara Y: Accumulation and aggregation of amyloid β -protein in late endosomes of Niemann-pick type C cells. *J Biol Chem* 2001, 276:4454–4460
10. Runz H, Rietdorf J, Tomic I, de Bernard M, Beyreuther K, Pepperkok R, Hartmann T: Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J Neurosci* 2002, 22:1679–1689
11. Burns M, Gaynor K, Olm V, Mercken M, LaFrancois J, Wang L, Mathews PM, Noble W, Matsuoka Y, Duff K: Presenilin redistribution associated with aberrant cholesterol transport enhances β -amyloid production in vivo. *J Neurosci* 2003, 23:5645–5649
12. Jin LW, Meezawa I, Vincent I, Bird T: Intracellular accumulation of amyloidogenic fragments of amyloid- β precursor protein in neurons with Niemann Pick type C defects is associated with endosomal abnormalities. *Am J Pathol* 2004, 164:975–985
13. Cataldo AM, Barnett JL, Pieroni C, Nixon RA: Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's disease: neuropathologic evidence for a mechanism of increased β -amyloidogenesis. *J Neurosci* 1997, 17:6142–6151
14. Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT, Nixon RA: Endocytic pathway abnormalities precede amyloid β deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. *Am J Pathol* 2000, 157:277–286
15. Cataldo AM, Petanceska S, Peterhoff CM, Terio NB, Epstein CJ, Villar A, Carlson EJ, Staufenbiel M, Nixon RA: App gene dosage modulates endosomal abnormalities of Alzheimer's disease in a segmental trisomy 16 mouse model of down syndrome. *J Neurosci* 2003, 23:6788–6792
16. Pentchev P, Vanier M, Suzuki K, Patterson M: Niemann-Pick disease type C: a cellular cholesterol lipidosis. *The Metabolic and Molecular Bases of Inherited Disease*. Edited by Scriver C, Beaudet A, Sly W, Valle D. New York, McGraw Hill, 1995, pp 2625–2639
17. Vanier MT, Millat G: Niemann-Pick disease type C. *Clin Genet* 2003, 64:269–281
18. Zervas M, Dobrenis K, Walkley SU: Neurons in Niemann-Pick disease type C accumulate gangliosides as well as unesterified cholesterol and undergo dendritic and axonal alterations. *J Neuropathol Exp Neurol* 2001, 60:49–64
19. German DC, Quintero EM, Liang CL, Ng B, Punia S, Xie C, Dietschy JM: Selective neurodegeneration, without neurofibrillary tangles, in a mouse model of Niemann-Pick C disease. *J Comp Neurol* 2001, 433:415–425
20. Ong WY, Kumar U, Switzer RC, Sidhu A, Suresh G, Hu CY, Patel SC: Neurodegeneration in Niemann-Pick type C disease mice. *Exp Brain Res* 2001, 141:218–231
21. Auer IA, Schmidt ML, Lee VM, Curry B, Suzuki K, Shin RW, Pentchev PG, Carstea ED, Trojanowski JQ: Paired helical filament tau (PHFtau) in Niemann-Pick type C disease is similar to PHFtau in Alzheimer's disease. *Acta Neuropathol (Berl)* 1995, 90:547–551
22. Suzuki K, Parker CC, Pentchev PG, Katz D, Ghetti B, D'Agostino AN, Carstea ED: Neurofibrillary tangles in Niemann-Pick disease type C. *Acta Neuropathol (Berl)* 1995, 89:227–238
23. Saito Y, Suzuki K, Nanba E, Yamamoto T, Ohno K, Murayama S: Niemann-Pick type C disease: accelerated neurofibrillary tangle formation and amyloid β deposition associated with apolipoprotein E ϵ 4 homozygosity. *Ann Neurol* 2002, 52:351–355
24. Tanaka J, Nakamura H, Miyawaki S: Cerebellar involvement in murine sphingomyelinosis: a new model of Niemann-Pick disease. *J Neuropathol Exp Neurol* 1988, 47:291–300
25. Loftus SK, Morris JA, Carstea ED, Gu JZ, Cummings C, Brown A, Ellison J, Ohno K, Rosenfeld MA, Tagle DA, Pentchev PG, Pavan WJ: Murine model of Niemann-Pick C disease: mutation in a cholesterol homeostasis gene. *Science* 1997, 277:232–235
26. Naureckiene S, Sleat DE, Lackland H, Fensom A, Vanier MT, Wattiaux R, Jadot M, Lobel P: Identification of HE1 as the second gene of Niemann-Pick C disease. *Science* 2000, 290:2298–2301
27. Carstea ED, Morris JA, Coleman KG, Loftus SK, Zhang D, Cummings C, Gu J, Rosenfeld MA, Pavan WJ, Krizman DB, Nagle J, Polymeropoulos MH, Sturley SL, Ioannou YA, Higgins ME, Comly M, Cooney A, Brown A, Kaneski CR, Blanchette-Mackie EJ, Dwyer NK, Neufeld EB, Chang TY, Liscum L, Strauss III JF, Ohno JF, Zeigler M, Carmi R, Sokol J, Markie D, O'Neill RR, van Diggelen DP, Elleder M, Patterson MC, Brady RO, Vanier MT, Pentchev PG, Tagle DA: Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. *Science* 1997, 277:228–231
28. Kobayashi T, Beuchat MH, Lindsay M, Frias S, Palmiter RD, Sakuraba H, Parton RG, Gruenberg J: Late endosomal membranes rich in lysobisphosphatidic acid regulate cholesterol transport. *Nat Cell Biol* 1999, 1:113–118
29. Nothurft A, Brown MS, Goldstein JL: Topology of SREBP cleavage-activating protein, a polytopic membrane protein with a sterol-sensing domain. *J Biol Chem* 1998, 273:17243–17250
30. Reaves BJ, Row PE, Bright NA, Luzio JP, Davidson HW: Loss of cation-independent mannose 6-phosphate receptor expression promotes the accumulation of lysobisphosphatidic acid in multilamellar bodies. *J Cell Sci* 2000, 113:4099–4108
31. Simons K, Gruenberg J: Jamming the endosomal system: lipid rafts and lysosomal storage diseases. *Trends Cell Biol* 2000, 10:459–462
32. Blom TS, Linder MD, Snow K, Pihko H, Hess MW, Jokitalo E, Veckman V, Syvanen AC, Ikonen E: Defective endocytic trafficking of NPC1 and NPC2 underlying infantile Niemann-Pick type C disease. *Hum Mol Genet* 2003, 12:257–272
33. Reid PC, Sugii S, Chang TY: Trafficking defects in endogenously synthesized cholesterol in fibroblasts, macrophages, hepatocytes, and glial cells from Niemann-Pick type C1 mice. *J Lipid Res* 2003, 44:1010–1019
34. Morell P, Jurevics H: Origin of cholesterol in myelin. *Neurochem Res* 1996, 21:463–470
35. Goodrum JF, Pentchev PG: Cholesterol reutilization during myelination of regenerating PNS axons is impaired in Niemann-Pick disease type C mice. *J Neurosci Res* 1997, 49:389–392
36. Mathews PM, Nixon RA: Setback for an Alzheimer's disease vaccine: lessons learned. *Neurology* 2003, 61:7–8
37. Mathews PM, Jiang Y, Schmidt SD, Grbovic OM, Mercken M, Nixon RA: Calpain activity regulates the cell surface distribution of amyloid precursor protein: inhibition of calpains enhances endosomal generation of β -cleaved C-terminal APP fragments. *J Biol Chem* 2002, 277:36415–36424
38. Grbovic OM, Mathews PM, Jiang Y, Schmidt SD, Dinakar R, Summers-Terio NB, Ceresa BP, Nixon RA, Cataldo AM: Rab5-stimulated up-regulation of the endocytic pathway increases intracellular β -cleaved amyloid precursor protein carboxyl-terminal fragment levels and A β production. *J Biol Chem* 2003, 278:31261–31268
39. Koo EH, Squazzo SL: Evidence that production and release of amyloid β -protein involves the endocytic pathway. *J Biol Chem* 1994, 269:17386–17389
40. Perez RG, Soriano S, Hayes JD, Ostaszewski B, Xia W, Selkoe DJ, Chen X, Stokin GB, Koo EH: Mutagenesis identifies new signals for β -amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including A β 42. *J Biol Chem* 1999, 274:18851–18856
41. Lange Y, Ye J, Rigney M, Steck T: Cholesterol movement in Niemann-Pick type C cells and in cells treated with amphiphiles. *J Biol Chem* 2000, 275:17468–17475
42. Cruz JC, Chang TY: Fate of endogenously synthesized cholesterol in Niemann-Pick type C1 cells. *J Biol Chem* 2000, 275:41309–41316
43. Karten B, Vance DE, Campenot RB, Vance JE: Trafficking of chole-

- terol from cell bodies to distal axons in Niemann Pick C1-deficient neurons. *J Biol Chem* 2003, 278:4168–4175
44. Mathews PM, Guerra CB, Jiang Y, Grbovic OM, Kao BH, Schmidt SD, Dinakar R, Mercken M, Hille-Rehfeld A, Rohrer J, Mehta P, Cataldo AM, Nixon RA: Alzheimer's disease-related overexpression of the cation-dependent mannose 6-phosphate receptor increases A β secretion: role for altered lysosomal hydrolase distribution in β -amyloidogenesis. *J Biol Chem* 2002, 277:5299–5307
45. Holtzman DM, Santucci D, Kilbridge J, Chua-Couzens J, Fontana DJ, Daniels SE, Johnson RM, Chen K, Sun Y, Carlson E, Allea E, Epstein CJ, Mobley WC: Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. *Proc Natl Acad Sci USA* 1996, 93:13333–13338
46. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL: Soluble pool of A β amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol* 1999, 46:860–866
47. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J: Soluble amyloid β peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol* 1999, 155:853–862
48. Kaye R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG: Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 2003, 300:486–489
49. Klein WL, Krafft GA, Finch CE: Targeting small A β oligomers: the solution to an Alzheimer's disease conundrum? *Trends Neurosci* 2001, 24:219–224
50. Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu S, Wu X, Holtzman DM, Paul SM: Immunization reverses memory deficits without reducing brain A β burden in Alzheimer's disease model. *Nat Neurosci* 2002, 5:452–457
51. Oster-Granite ML, McPhie DL, Greenan J, Neve RL: Age-dependent neuronal and synaptic degeneration in mice transgenic for the C terminus of the amyloid precursor protein. *J Neurosci* 1996, 16:6732–6741
52. McPhie DL, Golde T, Eckman CB, Yager D, Brant JB, Neve RL: β -secretase cleavage of the amyloid precursor protein mediates neuronal apoptosis caused by familial Alzheimer's disease mutations. *Brain Res Mol Brain Res* 2001, 97:103–113
53. Sopher BL, Fukuchi K, Smith AC, Leppig KA, Furlong CE, Martin GM: Cytotoxicity mediated by conditional expression of a carboxyl-terminal derivative of the β -amyloid precursor protein. *Brain Res Mol Brain Res* 1994, 26:207–217
54. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, St. George-Hyslop P, Westaway D: A β peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 2000, 408:979–982
55. Nixon RA: A "protease activation cascade" in the pathogenesis of Alzheimer's disease. *Ann NY Acad Sci* 2000, 924:117–131
56. Nixon RA, Cataldo AM, Mathews PM: The endosomal-lysosomal system of neurons in Alzheimer's disease pathogenesis: a review. *Neurochem Res* 2000, 25:1161–1172
57. Borsello T, Croqueolois K, Hornung JP, Clarke PG: N-methyl-d-aspartate-triggered neuronal death in organotypic hippocampal cultures is endocytic, autophagic, and mediated by the c-Jun N-terminal kinase pathway. *Eur J Neurosci* 2003, 18:473–485
58. Koike M, Nakanishi H, Saftig P, Ezaki J, Isahara K, Ohsawa Y, Schulz-Schaeffer W, Watanabe T, Waguri S, Kametaka S, Shibata M, Yamamoto K, Kominami E, Peters C, von Figura K, Uchiyama Y: Cathepsin D deficiency induces lysosomal storage with ceroid lipofuscin in mouse CNS neurons. *J Neurosci* 2000, 20:6898–6906
59. Cooper JD, Salehi A, Delcroix JD, Howe CL, Belichenko PV, Chua-Couzens J, Kilbridge JF, Carlson EJ, Epstein CJ, Mobley WC: Failed retrograde transport of NGF in a mouse model of Down's syndrome: reversal of cholinergic neurodegenerative phenotypes following NGF infusion. *Proc Natl Acad Sci USA* 2001, 98:10439–10444
60. Cooper JD: Progress towards understanding the neurobiology of Batten disease or neuronal ceroid lipofuscinosis. *Curr Opin Neurol* 2003, 16:121–128
61. Orth M, Mundegar RR: Effect of acid maltase deficiency on the endosomal/lysosomal system and glucose transporter 4. *Neuromuscul Disord* 2003, 13:49–54
62. Chen B, Borinstein SC, Gillis J, Sykes VW, Bogler O: The glioma-associated protein SETA interacts with AIP1/Alix and ALG-2 and modulates apoptosis in astrocytes. *J Biol Chem* 2000, 275:19275–19281
63. Trioulier Y, Torch S, Blot B, Cristina N, Chatellard-Causse C, Verna JM, Sadoul R: Alix, a protein regulating endosomal trafficking, is involved in neuronal death. *J Biol Chem* 2004, 279:2046–2052
64. Gout I, Middleton G, Adu J, Ninkina NN, Drobot LB, Filonenko V, Matsuka G, Davies AM, Waterfield M, Buchman VL: Negative regulation of PI 3-kinase by Ruk, a novel adaptor protein. *EMBO J* 2000, 19:4015–4025
65. Yu W, Cuervo A, Kumar A, Schmidt S, Cataldo A, Mathews P, Nixon R: Autophagy and its role in amyloid precursor protein processing. Presented at the Society for Neuroscience, New Orleans LA, 2003, 12:2003 (Abstract)
66. Delcroix JD, Valletta JS, Wu C, Hunt SJ, Kowal AS, Mobley WC: NGF signaling in sensory neurons: evidence that early endosomes carry NGF retrograde signals. *Neuron* 2003, 39:69–84
67. Ikin AF, Annaert WG, Takei K, De Camilli P, Jahn R, Greengard P, Buxbaum JD: Alzheimer amyloid protein precursor is localized in nerve terminal preparations to Rab5-containing vesicular organelles distinct from those implicated in the synaptic vesicle pathway. *J Biol Chem* 1996, 271:31783–31786
68. Marquez-Sterling NR, Lo AC, Sisodia SS, Koo EH: Trafficking of cell-surface β -amyloid precursor protein: evidence that a sorting intermediate participates in synaptic vesicle recycling. *J Neurosci* 1997, 17:140–151
69. Kamal A, Stokin GB, Yang Z, Xia CH, Goldstein LS: Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I. *Neuron* 2000, 28:449–459