Research Progress Report

2/3/2006 Nobutaka Ohgami (TY Chang's laboratory)

Title: Demonstration of NPC1 as a lipid binding protein *in Vitro*

Lay summary

This present study aims at testing the possibility that NPC1 may directly bind cholesterol and/or other lipid. First, in vitro (cell free) photolabeling of NPC1-YFP protein with both ^{3}H-labeld 3□α-ol isomer (= ^{3}H-AC-3□α-ol) and 3β-ol isomer (= ^{3}H-AC) of photoactivatable cholesterol analog, 7,7-azocholestanol showed that ^{3}H-AC-3□α-ol labeled NPC1-YFP protein much less efficiently than that ^{3}H-AC suggesting that 3-beta OH moiety of the steroid ring A plays an important role in the direct interaction between the NPC1 protein and AC. Next, the pulse and chase experiment with [^{3}H]cholesteryl linoleate-labeled low-density lipoprotein ([^{3}H]CL-LDL) showed that the sterol-sensing domain (SSD) mutants (Y635C and P692S) are loss of function mutants. Finally, the preliminary result showed that the SSD mutant (Y635C) obviously diminished the extent of the labeling compared to WT, suggesting at least Y635 within the SSD of NPC1 could be responsible for the sterol binding of this protein in the cell free condition. In the future, I will further perform this cross-linking assay by including the SSD mutant (P692S) as well.