Stony Brook University
The Graduate School

Doctoral Defense Announcement

Abstract

Functional Dissection of the Proteins of the Endoplasmic Reticulum Associated Degradadtion (ERAD) Pathway

By

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Newly synthesized glycoproteins acquire their "folded" conformation by concerted action of chaperones in the lumen of the endoplasmic reticulum (ER). Glycoproteins that fail to fold correctly are transported to the cytosol and are eventually degraded by the 26S proteasome, by a process known as ER associated degradation (ERAD). My dissertation evaluates the role of three proteins, namely peptide: N-glycanase (PNGase), gp78 and Derlin-1, in the ERAD process.

PNGase is a cytosolic deglycosylating enzyme that cleaves N-linked glycans. By developing an *in vitro* assay, I have shown that, a) PNGase deglycosylates full-length glycoproteins and not just glycopeptides as previously believed; b) glycoprotein misfolding is a prerequisite for PNGase mediated action. My findings on the subcellular localization of PNGase in HeLa cells show that a minor fraction of PNGase is tethered to the ER via Derlin-1, an ER membrane protein. This interaction with Derlin-1, which presumably functions in glycoprotein dislocation from the ER, brings PNGase in close proximity to misfolded glycoproteins.

Gp78, also known as autocrine motility factor receptor, was originally identified as a cell-surface glycoprotein important in tumor cell motility. Subsequently gp78 was found to function as an ER associated E3 ubiquitin-ligase involved the turnover of misfolded glycoproteins. Using a combinatorial approach of biotinylation and confocal microscopy, I have shown that gp78 localizes exclusively to the ER. Also, my analysis of gp78 using the deglycosylating enzyme Endo H refutes the notion that this protein is modified by N-linked glycans, due to the lack of it's mobility shift on SDS-PAGE upon Endo H treatment.

The Derlin family of ER membrane proteins, comprised of Derlin-1, 2 and 3 in humans have been recently identified as ERAD factors involved in the dislocation of misfolded glycoproteins. To date, only four substrates of the Derlins have been identified: CPY*, MHC class I heavy chains, CFTR and α-1 antitrypsin. I have identified a novel substrate of Derlin-1: CD3-δ, a component of the T cell receptor complex. My data suggests that Derlin-1 interacts with CD3-δ and Derlin-1 overexpression in HeLa cells causes its accelerated turnover. These findings suggest the importance of Derlin-1 in the pathway for CD3-δ degradation.

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