

Role of EDEM in the Release of Misfolded Glycoproteins from the Calnexin Cycle

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The mechanisms that determine how folding attempts are interrupted to target folding-incompetent proteins for endoplasmic reticulum-associated degradation (ERAD) are poorly defined. Here the α -mannosidase I-like protein EDEM was shown to extract misfolded glycoproteins, but not glycoproteins undergoing productive folding, from the calnexin cycle. EDEM overexpression resulted in faster release of folding-incompetent proteins from the calnexin cycle and earlier onset of degradation, whereas EDEM down-regulation prolonged folding attempts and delayed ERAD. Up-regulation of EDEM during ER stress may promote cell recovery by clearing the calnexin cycle and by accelerating ERAD of terminally misfolded polypeptides.

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Summary of the scientific background:

In cells, the endoplasmic reticulum (ER) is the compartment responsible for the synthesis of all secreted proteins (hormones, antibodies, pancreatic enzymes, etc), all membrane proteins present on the cell surface (receptors, channels, etc) and all proteins residing in intracellular compartments such as the lysosomes. For proper functioning, a protein folds in a characteristic three-dimensional structure and may form a multimeric protein complex. The ER provides an environment optimized for the folding and assembly of proteins.

Efficient and rapid protein folding is assisted by molecular chaperones, folding factors and folding sensors. Chaperons associate with novel proteins and expose them to folding factors, which mediate critical steps of the protein folding. As an example, they stabilize the structure of proteins with the formation of disulfide bonds. The folding sensors determine if proteins have the correct structure. Only then, the transport of proteins to their final destination can happen. If the correct structure has not been acquired, the protein is recycled and the folding attempt is repeated, a process defined as the calnexin cycle.

A deployment of malfunctioning proteins might be detrimental to the organism. The quality control system operating in the ER ensures the fidelity of the maturation process by blocking the delivery of incompletely folded, incompletely assembled and misfolded proteins. Terminally misfolded proteins are cleared in the cytosol by processes collectively defined as ER-associated degradation (ERAD). The mechanisms determining how unsuccessful folding attempts are interrupted and proteins are degraded are poorly defined.

In this paper the authors report that the ER-resident mannose-binding lectin EDEM regulates the retention in the ER of glycoproteins with aberrant folding. When the concentration of EDEM is increased in the cell, folding-incompetent proteins are released earlier from the calnexin cycle and are degraded rapidly. On the contrary, a lower amount of EDEM prolongs folding in the calnexin cycle and ERAD is delayed. The authors conclude that a cell may up-regulate EDEM, a known stress-induced gene, in response to the accumulation of misfolded proteins in the ER caused by certain pathological situations.

The ER is the intracellular organelle responsible for proper maturation of newly synthesized proteins. The ER-resident lectin EDEM ensures that folding-incompetent or terminally misfolded glycoproteins are rapidly cleared from the ER lumen. The mechanisms described in this paper may be essential to prevent processes, which may ultimately lead to cell death through intracellular accumulation of malfunctioning proteins.

Relevance of data reported:

An increasing number of strongly debilitating human diseases has been ascribed to defective protein folding and quality control. Examples are cystic fibrosis, hereditary lung emphysema, diabetes, familial hypercholesterolemia, melanoma, retinitis pigmentosa and neurodegenerative diseases. In the so called "conformational diseases" mutated proteins that do not acquire their native structure are diverted to ERAD. If their disposal is not efficient, they might accumulate intra- or extracellularly triggering severe phenotypes and damage the tissue.

Pathogens may also exploit the host ERAD machinery to escape immunosurveillance. As an example, the cytomegalovirus or Epstein-Barr virus trigger the degradation of MHC class I antigens which are required by the immune system to identify infected cells. Also bacterial and plant toxic agents such as cholera and ricin toxins may use components of the ERAD machinery to invade the inside of the cell. The elucidation of the cellular pathway regulating protein folding and quality control opens the possibility to interfere with said pathological processes.

Alzheimer's disease is a progressive dementia characterized by the deposition of β -amyloid plaques in the brain. It is expected that interfering with the production of β -amyloid might represent a pharmacologic intervention for the treatment of the disease. In the brain, the enzyme BACE initiates the processing pathway that produces β -amyloid. The authors report the existence of a human form of this enzyme that due to a defect in protein folding is completely inactive. This might be a mechanism used by cells to regulate the enzymatic activity of BACE and the production of β -amyloid. The results give more detailed insights in pathways which may lead to Alzheimer's disease.

Glossary

What is an ER?

The endoplasmic reticulum (ER) is a series of interconnected, intracytoplasmic, membrane bounded sacs. The term "endo" refers to within the cytoplasm and a reticulum is a network. The ER plays an important part in the synthesis of lipids and proteins, which are destined to be inserted in the cell membrane. There are two types of ER: smooth and rough.

Smooth ER is the site of synthesis and digestion of fatty acids and phospholipids. In the liver it is used to modify dangerous chemicals such as pesticides ready for excretion.

Rough ER is the site of manufacture of secretory proteins as well as proteins destined to be inserted in the cell membrane. It is rough because of the vast number of ribosomes which stud its surface. Proteins synthesized by these ribosomes are injected into the lumen of the ER. Folding and assembly of the newly synthesized polypeptides starts already during their synthesis, and it is assisted by molecular chaperones and folding factors resident in the ER.

What does ERAD mean?

There are several "quality control" mechanisms to ensure that only correctly folded proteins and completely assembled polypeptides are exported from the ER. Terminally misfolded proteins are transported into the cytosol and degraded in processes collectively defined as ER-associated degradation (ERAD).

What is a chaperone?

Molecular chaperones are present in the ER and in the cytosol of all cells. In the ER, the chaperones stick to immature, but also to misformed proteins, and assist them during folding. Molecular chaperones also ensure that non-native polypeptides leave the ER and are degraded. Genetic mutations can prevent some protein to achieve the correct conformation. These misfolded proteins are not released from the chaperones and accumulate inside the ER, inside the cytosol or at the surface of the cells if the degradation machinery is damaged or overwhelmed. For example cystic fibrosis is caused by a build up of misformed proteins, together with their chaperones, inside ER vesicles. Other illnesses thought to be connected to misfolded proteins include hereditary lung emphysema, diabetes, familial hypercholesterolemia, melanoma, retinitis pigmentosa, neurodegenerative diseases.

What is the calnexin cycle?

One of the most common modifications of proteins expressed into the ER is the addition of sugars (N-linked glycans). For glycoproteins, a particularly well studied ER quality control system is in place, involving two homologous lectins, calnexin and calreticulin. Calnexin is a transmembrane protein and calreticulin is a soluble protein retained in the ER lumen by a C-terminal retention signal.

Calnexin and calreticulin associate with glucose residues on N-linked oligosaccharides attached to newly synthesized glycoproteins. The newly synthesized glycoprotein is thus folded while attached to calnexin and calreticulin. It is released from the two lectin-chaperones and exits the ER when folding has been completed.

Cooperation of the Institute for Research in Biomedicine, Bellinzona with Novartis Institute for Biomedical Research NIBR:

The group of Dr. Molinari at the IRB in Bellinzona started a co-operation with the group of Dr. Paganetti at the Novartis Institutes for Biomedical Research Basel at the beginning of 2000. The aim of the collaboration is the analysis of the mechanisms of protein folding, quality control and ER-associated degradation using as a model proteins involved in Alzheimer disease, one of NIBR's main fields of research. Novartis runs a purely non-commercial, informal academic cooperation with the Research Institute in Bellinzona. For more details on Novartis collaborations in R&D please refer to

<http://www.novartis.com/investors/en/alliances.shtml>