

Co-Translational Insertion

Sequence of Events: Sorting Newly Synthesized Protein

- Chapter 8 in the book
- Goblet cell found in the wall of the intestine
 - To secrete proteins that have protective roles
 - Mucigens are complexes of proteins and carbohydrates, long chains of sugar
 - Will coat the surface of the small intestine (like mucus) to protect the cells that line the intestine from the digestive enzymes functioning in this compartment
 - Help lubricate the movements of material passing through intestine to minimize friction
 - Mucigens secrete these glycoproteins for these reasons
 - Structure of cell is interesting
 - Lumen of intestine where secretion takes place is at the top
 - Secretion occurs, secretion triggered, these vesicles have a lipid bilayer and material secreted across vesicle, across plasma membrane into other cell
 - Each cell has a nucleus, mitochondria, Golgi complex (green), rough ER and other membranes (peroxisomes, lysosomes)
- These classes of cells are known as polarized cells
 - Structurally one end of the cell is different than the other
 - Nucleus is at one end, secretory granules are at the other end
 - *Location of the ER and the Golgi are quite distinct
 - *Unique compartments
 - What exists in the secretory vesicle is quite different compared to protein composition in the nucleus, which is different than what you find in the mitochondria, etc.
 - Fundamental question: how does this sorting take place?
- Proteins in these cells can be made at one of 3 sites
 - Cytoplasm
 - ER
 - Mitochondria
 - Sorting has to be the consequence of its formation that exists somewhere
- Start from as soon as these cells begin to be resolved by EM, cell biologists interested in how to sort assembly of all of these proteins – led to Nobel prizes
 - George Palade at the Rockefeller Center on sorting the route of assembly of proteins
 - 25 years later his students won another Nobel prize for molecular mechanism for these protein (Hunter)

Sorting Newly-Produced Proteins: Co-Translational

- Pancreatic Model
 - Most important model used, very useful
 - Look for subset of proteins destined for secretory vesicles
- Assembly of Secretory Vesicles
- Signal Sequences
- Integral Membrane Proteins
 - Signal sequencing and integral membrane proteins led to the 2nd Nobel prize (Hunter and ?)
- Core Glycosylation
 - Look at how they get their sugars added to them, what specifically is added where and when
- Sorting Lysosomal Enzymes

Pancreatic Acinar Cell

- Different classes of cell in the pancreas are localized in different regions
 - Alpha cells which secrete the hormone glucagon
 - Beta cells are involved in insulin secretion
 - Acinar cells which secrete digestive enzymes
 - Enzymes get delivered to the intestine after a meal (around the stomach in the digestive “travel”) hormone is released (peptide hormone from cells and is triggered to release digestive enzymes and then these enzymes are released inside a duct tube and duct leads to small intestine where digestion is complete
 - Think of these acinar cells as lining a duct
 - Line up duct – nuclei are at the bottom, secretory vesicles are brown and at the top of cell will sit next to the membrane of those cells that are facing the duct (secretion occurs that way)
 - Describes orientation of cells
- Pancreas is located to the left in the abdomen and next to the stomach
- Look at cells under EM, secretory vesicles are surrounded by a lipid bilayer and contains enzymes along with nucleus, rough ER and Golgi sitting next to nucleus
 - There are vesicles that sit between organelles and secretory vesicles are known as intermediate vesicles
- When cells are stimulated by peptide hormone, there are calcium changes in cells and vesicle can fuse with plasma membrane
 - Known as a stimulated secretion
- Pancreatic acinar cells were very useful
 - Most of the proteins that they synthesize end up going into the secretory vesicle
 - Protein being made – more likely that the protein is destined to be isolated in the secretory vesicle
 - Can take slices of the pancreas and if the cells are incubated in the right buffer, cells will continue to survive and produce proteins

- Can do animal freeing experiments
 - These two traits – cells are primarily involved in the production of secretory proteins
 - Can survive for significant period in animal-free environment and continue to function (very useful)
- Given structure of cells, where are these proteins are made?
- Simple experiment, profound results = where are proteins made that end up in these vesicles?
 - 3 classes of ribosomes of eukaryotic cells

Protein Synthesis in Eukaryotes

- Read page 350-370 on protein synthesis (visualize sequence of events in cell)
- mRNA that has a 3' and 5' end in eukaryotic cell that will be polyadenylated
 - 3' has capsulated structure
 - Ribosomes begin by some of the subunits associating with the 5' end of the messenger RNA and begin to translate the protein
 - Ribosomes move along messenger and begin to form polypeptide
 - Eventually dangle from a tunnel in the middle of the large ribosomal subunit
 - tRNAs deliver amino acid to the messenger RNA segment where you have a codon being used
 - As ribosomes move, segment of polypeptides become longer and longer as ribosome progressively moves toward 3' end
 - Starts at the amino end of the protein is the one that is made first – very important

ER Ribosomes

- Location of ribosomes at 3 sites
 - Some in mitochondria and chloroplasts – synthesis of some protein
 - Translation of majority of proteins occurs
 - In free ribosomes – float in cytoplasm
 - Ribosomes associated with rough endoplasmic reticulum (ER bound ribosomes)
 - 2 target candidate sites for the assembly of secretory proteins
- Ribosomes associated with the ER
 - 2 types of ER includes the rough ER and smooth ER
 - ER membrane is made up of lipid bilayers
 - When ribosomes are associated with the ER – large ribosomal subunits sit on the membrane
 - Functions of rough ER
 - Synthesis of proteins
 - Function of smooth ER
 - Compartment that stores calcium in some cells
 - Involved in detoxification
 - Liver enzymes involved in degrading a variety of enzymes