

MOLECULAR CELL BIOLOGY: ESSAY OUTLINE

What are peroxisomes? What do they do? And, how are proteins targeted to them?

Are they related to the ER/Golgi? Are they more like mitochondria? Or, are they completely different again? Include a discussion of what happens to cells and organisms if peroxisomes are defective.

I. INTRODUCTION

- **Briefly introduce what a peroxisome is and its situation in the body.**
 - Vesicles within every cell containing oxidative enzymes
- **Describe its theorised History including its early functions, development and replacement by mitochondria.**
 - Early function - *Originates from an “ancient organelle” that performed all oxygen metabolism. [ref 1]*
 - Development - *At the time, most cells had an anaerobic metabolism so oxygen produced by photosynthetic bacteria was toxic, peroxisomes aimed to reduce the levels of oxygen but did not produce energy from it as mitochondria does. [ref 1]*
 - Replacement – *Mitochondria took over most functions of the peroxisomes, most importantly coupling reactions involving oxygen with ATP formation. Peroxisomes were then only useful for the reactions that mitochondria could not do. [ref 1]*
- **Describe briefly its function in modern day life.**
 - *Function – “oxidation of fatty acids, biosynthesis of cholesterol, bile acids and plasmalogen, and detoxification of H₂O₂” [ref 3]*
- **Introduce the idea that its malfunction can cause diseases such as Zellweger syndrome**
 - *although once thought to be an organelle of little function it still remains a vital part of the cell, defects/mutations in peroxisomes can lead to severe neurological disorders such as Zellweger Syndrome, rhizomelic chondrodysplasia punctata and X-chromosome-linked adrenoleukodystrophy. [ref 1,3]*

II. STRUCTURE

- **Shape and size**
 - *spherical structure [ref 6]*
 - *0.1µm – 1.0µm [ref 6]*
- **Membrane**
 - *single phospholipid bilayer [ref 1]*
- **Core**
 - *granular matrix [ref 6]*
 - *sometimes paracrystalline core [ref 6]*
 - *Contain many enzymes which are unique to the different cell types within the body [ref 1]*
- **No DNA + no ribosomes**
 - *Therefore have to acquire all proteins via selective import [ref 1]*

- *Able to import fully folded and oligomeric proteins (distinguishes it from mitochondria and ER that cant) [ref 7]*
- **Peroxisomal biogenesis - peroxins:**
- *proteins required for peroxisomal assembly, they are encoded by PEX genes. [ref 5]*
- *“To date 32 peroxins are known, and they are involved in the three key stages of peroxisomal development: (i) formation of the peroxisomal membrane, (ii) peroxisome proliferation and (iii) compartmentalization of peroxisomal matrix proteins.” [ref 7]*
- *Formed from new at the ER but can self replicate once formed [ref 7]*

- **Comparison with other structures:**

1. MITOCHONDRIA

- **Shape and size**

- *Oblong*
- *0.5 μ m – 1 μ m [ref 1]*

- **Membrane**

- *outer membrane – consists of transport proteins (porin), can import small intact proteins [ref 1]*
- *inner folded membrane – respiratory chain enzymes embedded here, perform oxidative phosphorylation [ref 1]*

- **Core**

- *Internal matrix [ref 1]*
- *Carries out β – oxidation [ref 1]*
- *Glycolysis which produces ATP and uses oxygen [ref 1]*
- *Intermembrane space [ref 1]*
- *contains several enzymes [ref 1]*

- **Its own DNA and ribosomes**

- *so it acquires only some of its proteins via selective import*

- **Self replicating**

- **Mitochondrial-peroxisomal complementation** – *knockout mouse developed by disrupting Pex5 gene [ref 4]*

- *Caused – a change in the expression and activities of respiratory chain enzymes in the mitochondria, increased the volume of oxygen present in the cell possibly due to the lack off peroxisomal oxidation reactions occurring, increased the number of fatty acid intermediates from β -oxidation. [ref 4]*
- *Effect – mouse showed severe defects in peroxisomal uptake of proteins, lack in functioning peroxisomes, exhibited all major signs of Zellweger syndrome patients. [ref 4]*
- *Conclusion – “absence of functional peroxisomes leads to severe alterations in mitochondrial ultrastructure.” [ref 4], therefore peroxisomes do reactions that the mitochondria cannot do.*

2. ENDOPLASMIC RETICULUM

- **Membrane**

- *single membrane*

- **Does not have its own unique DNA**

- **Self-replicating**

- **Evidence that peroxisomes originate from the ER**

- *PEX3p initially located on ER that is required for the budding process [ref 7]*
- **ER forms new peroxisomes but peroxisomes can undergo budding once formed.** [ref 7]

III. PROTEIN IMPORT

- **Describe why protein import must take place**
 - *because it lacks its own DNA or indeed any of the required machinery it cannot produce its own proteins.* [ref 1]
- **Insertion of peroxisomal membrane proteins (PMPs)**
 - *Requires Pex3p + Pex19p* [ref 7]
 - *PMPs have a peroxisomal membrane targeting signal (mPTS)* [ref 7]
 - *Pex19p (which behaves as an import receptor and chaperon) recognises and binds to mPTS on PMP and delivers its PMP protein to the membrane. This occurs at ER (during formation of new peroxisomes) as well as at the peroxisomal membrane.* [ref 7]
 - *“Pex3p functions as membrane anchor protein for the Pex19p-PMP complexes at the ER and peroxisome membrane.”* [ref 7]
- **Describe how these proteins are recognised**
 - *a specific sequence of 3 amino acids (Ser-Lys-Leu-COO-) at the C or N terminus must be present called the PTS (peroxisomal targeting signal sequence).* [ref 2]
 - *Proteins with PTS 1 have a “carboxy terminal tripeptide” sequence* [ref 3]
 - *Proteins with PTS 2 have a recognisable sequence at the N-terminus, 11-16 amino acids long* [ref 3]
 - *If this signal sequence is added to a protein not required by the peroxisome it will still be imported –* [ref 2]
- **Describe the process of importation:**
 - *Soluble receptor proteins (encoded on Pex5 [ref 4]) in the cytosol of the cell recognise and bind to the desired protein to be imported into the peroxisome and transport it to the peroxisomal membrane.* [ref 7]
 - *Soluble receptor Pex5p recognises proteins exhibiting PTS1* [ref 7]
 - *Soluble receptor Pex7p recognises proteins exhibiting PTS2* [ref 7]
 - *Docking complex – binding site on peroxisomal membrane at which the receptor-protein complex binds.* [ref 7]
 - *Receptor-protein complex transported across the membrane into the peroxisome.* [ref 7]
 - *Receptor releases protein* [ref 7]
 - *Receptor exits the peroxisome* [ref 7]

IV. FUNCTION

- **Describe the function of the enzymes:**
 - *They contain several oxidising enzymes that remove hydrogen from compounds using oxygen which produces H₂O₂ as a toxic by-product.*
 - *Catalase – “uses the H₂O₂ produced in the peroxisome to oxidise other substrates (incl. phenols, formic acid, formaldehyde and alcohol) via the peroxidative reaction – H₂O₂ + RH₂ → R + 2H₂O”* [ref 1]
 - *Urateoxidase- oxidises uric acid*

- **Detoxification**
 - *The peroxidative reaction is used to detoxify toxins that enter the blood (specifically in the kidney and liver) such as ethanol to acetaldehyde [ref 1]*
- **β – oxidation**
 - *the progressive shortening of long fatty acid chains by the release of a two carbon compound, acetyl CoA, which is released into the cytosol for metabolic processes such as respiration. [ref 1]*
- **Plasmalogen synthesis**
 - *Plasmalogens are the most common phospholipids in myelin. “Peroxisomes catalyze the first reactions in the formation of plasmalogens.” [ref 1] If this process is inhibited in any way then the myelination of nerve cells cannot occur properly which leads to the development of neurological disorders. [ref 1] “Plasmalogens make up 80% of the white matter in brains.” [ref 7]*

V. PEROXISOMAL DISORDERS

- **Peroxisomal disorders identified so far have been put into two categories: [ref 3]**
- **Group one:**
 - *“have specific functional defects due to an abnormality in its DNA sequence” (e.g. X chromosome-linked adrenoleukodystrophy) [ref 3]*
- **Group two:**
 - *“multiple defects divided into 2 subgroups”*
 - a) intact peroxisomes (e.g. rhizomelic chondrodysplasia punctata), and*
 - b) lack peroxisomes (e.g. Zellweger syndrome) [ref 3]*
- **Causes of defects:**
 - *Defects in PEX genes disturb the process of protein importation from the matrix (in most cases) [ref 5]*
- **Zellweger syndrome**
 - *Inherited disorder*
 - *Enlarged liver, high levels of iron and copper in blood [ref 8]*
 - *Caused by a defect in PXR1 gene (Pex receptor 1 – a receptor required for the importation of proteins/enzymes into peroxisomes) [ref 8]*
- **Adrenoleukodystrophy (ALD)**
 - *Inherited disorder, rare [ref 9]*
 - *Loss of myelination on nerve fibres in the brain (i.e. deficiency in plasmalogen biosynthesis[ref 10]), accumulation of long chain fatty acids in brain and adrenal cortex, degeneration of adrenal gland [ref 9]*
 - *Caused by – mutation in PTS1 receptor and so peroxisomes are deficient in PTS 1 linked proteins [ref 3]*
- **Rhizomelic chondrodysplasia punctata (RCDP)**
 - *“severe symmetrical shortening of upper extremities”, stunted growth, cataract [ref 10]*
 - *Caused by – mutation in PTS2 receptor, thus peroxisomes are deficient in proteins displaying PTS2 [ref 3], leading to deficiency in plasmalogens, defective import of peroxisomal thiolase, however β –oxidation is functioning normally [ref 10]*

- **Catalase import study** – peroxisomes expressing normal packaging of PTS 1 and PTS 2 proteins, but lack catalase exhibit multiple enzymatic dysfunctions. [ref 3]

Procedure

part i) mutant cells were transfected with normal catalase with a) an attached PTS1 and b) an attached PTS 2 sequence [ref 3]

Part ii) “Normal cells were treated with aminotriazole to inhibit catalase” [ref 3]

Part iii) mutant cells were targeted with catalase attached to a) PTS 1 and b) PTS 2 [ref 3]

- Results

- Part i) reintroduction of catalase restored the peroxisomes functions – levels of fatty acids and plasmalogens returned to normal [ref 3]

- Part ii) “peroxisomal functions were inhibited to the level found in mutant cells” [ref 3]

- Conclusions

- the multiple enzyme dysfunctions are due to their inactivation due to the increase of H₂O₂ usually broken down by catalase. [ref 3]

- part iii) suggests there is an alternative pathway for the uptake of catalase and an “abnormality in this pathway manifests as a peroxisomal disease” [ref 3]

VI. Conclusion

• **What are peroxisomes?**

- Essential compartments within cells, vital for normal functioning
- Disruption of peroxisomes effects nerves, essential metabolic pathways and the functioning of mitochondria

• **How do they compare with other ER and mitochondria?**

- May have been taken over by mitochondria but did and do not originate from them
- Produced from new by the ER so similarities in some membrane proteins occur.

• **Overall?**

- Not the redundant organelle once speculated about.

References:

1. ALBERTS, B., et al, 2002. *Molecular biology of the cell*, 4th Edition, New York, Garland Science.
2. BERG, J. M., et al, 2002. *Biochemistry*, 5th Edition, New York, W. H. Freeman.
3. SHEIKH, F. G., et al, 1998. *Abnormality in catalase import into peroxisomes leads to severe neurological disorder*, Charleston, The National Academy of Sciences. [online] Available from: <http://ukpmc.ac.uk/articlerender.cgi?tool=pubmed&pubmedid=950119>. Accessed: 19/10/07
4. Mitochondrial Alterations caused by defective peroxisomal biogenesis in a mouse model for Zellweger syndrome (pex5 knockout mouse)
5. MUNTAU, A. C., et al, 2000. *Defective Peroxisome Membrane Synthesis Due To Mutations in Human PEX3 Causes Zellweger*

- Syndrome, Complementation Group G* [online]. The American Society of Human Genetics. Available from:
<http://ukpmc.ac.uk/articlerender.cgi?tool=pmcentrez&accid=pmcA1287898#IDAZCU2#IDAZCU2> (Accessed 24/10/07)
6. BASCOM, R.A., et al, 2003. *Peroxisome Biogenesis Occurs in an Unsynchronized Manner in Close Association with the Endoplasmic Reticulum in Temperature-sensitive Yarrowia lipolytica Pex3p Mutants* [online]. Alberta, Vol. 14, Issue 3, P939-957. Available from:
<http://www.molbiolcell.org/cgi/content/abstract/14/3/939> (Accessed 24/10/07)
 7. PLATTA, H. W., ERDMANN, R., 2007. *The peroxisomal protein import machinery*. [online] Elsevier B.V., Volume 581, Issue 15, Pages 2811-2819 (Vienna Special Issue: Molecular Machines), available from:
http://www.sciencedirect.com.ezproxy.sussex.ac.uk/science?_ob=ArticleURL&_udi=B6T36-4NFRYRN-3&_user=128860&_coverDate=06%2F19%2F2007&_rdoc=1&_fmt=&_orig=search&_sort=d&_view=c&_acct=C000010638&_version=1&_urlVersion=0&_userid=128860&_md5=f7b8ae19be9c689f96737862a0410d40#sec1 (Accessed 24/10/07)
 8. NCBI, 2007. *Zellweger syndrome* [online]. Available from:
<http://www.ncbi.nlm.nih.gov/disease/Zellweger.html> Accessed: 24/10/07
 9. NCBI, 2007. *Adrenoleukodystrophy* [online]. Available from:
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=gnd.section.226&ref=sid_ebar. Accessed: 24/10/07
 10. SIEGAL, G. J., 1999. *Peroxisomal disease* [online]. American Society for Neurochemistry, Lippincott Williams and Wilkins. Available from:
<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?highlight=Rhizomelic%20chondrodysplasia%20punctata&rid=bnchm.section.2913#2915> Accessed: 24/10/07