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Comparative analysis of highly purified rough endoplasmic reticulum by advanced proteomic technologies, 2D DIGE (16-BAC/SDS & IEF/SDS) and LC-MS/MS

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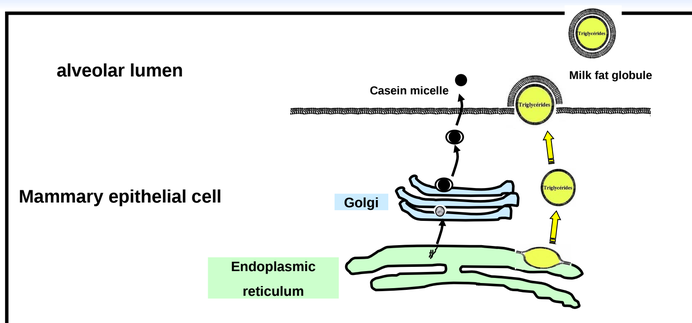
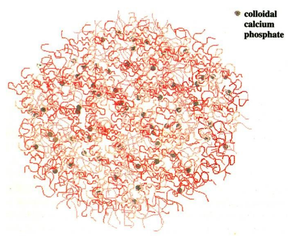
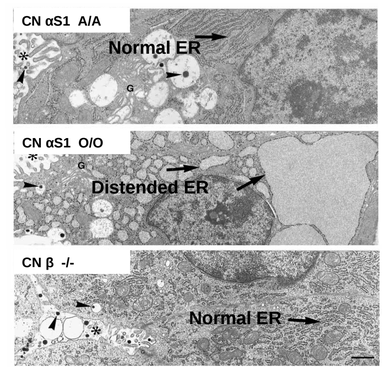
Background

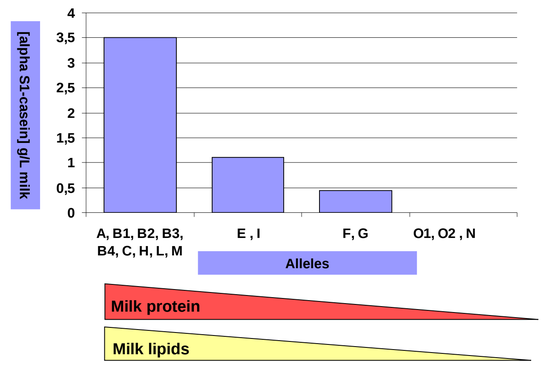




Casein micelle

During lactation, mammary epithelial cells synthesize and secrete huge quantities of milk specific proteins, lipids and lactose. The biogenesis of milk supramolecular structures, casein micelles and milk fat globules, is initiated in the endoplasmic reticulum (ER) of mammary epithelial cells. These components then follow independent intracellular trafficking pathways and milk supramolecular structures are released into the alveolar lumens of the mammary gland.





The lack of CSN1S1 in goat induces the accumulation of the other caseins in the ER. In addition, the secretion of both milk proteins and lipids was shown dramatically affected in the milk of defective goat.

Materials and Methods

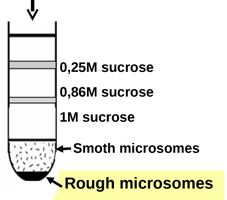
Purification of rough microsomes

))Adapted from Paiement et al. 2004

Homogenate of goat mammary tissues

Differential centrifugation

Sucrose gradient



Rough microsomes

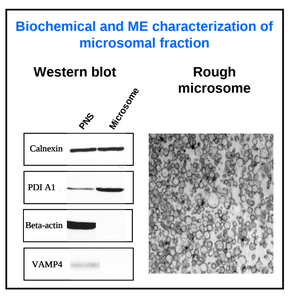
Saponin treatment

Three freeze-thawing cycles

Bicarbonate buffer pH 11

Microsomal membrane proteins

Biochemical and ME characterization of microsomal fraction



2D DIGE analysis

Microsomal membrane proteins

Rough microsome

2D DIGE 16-BAC / SDS PAGE

2D DIGE IEF / SDS PAGE

(3-10 NL and 4-7 L)

2D DIGE: Minimal labelling of proteins

Cyanine / Pair	1	2	3	4	5
Cy3	Goat117 A/A	Goat558 O/O	Goat70 A/A	Goat80 O/O	Goat93 A/A
Cy5	Goat152 O/O	Goat533 A/A	Goat71 O/O	Goat55 A/A	Goat76 O/O
Cy2 (Internal standard)	Pool	Pool	Pool	Pool	Pool

400pmol CyDyes/50µg proteins

The internal standard contents equal amount of all goat samples

LC-MS/MS analysis of tryptic digest of samples

Nano Liquid chromatography

Ultimate 3000 (Dionex)

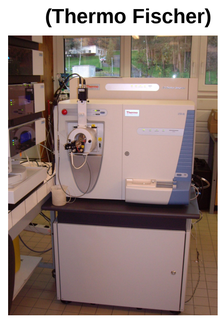
Nano Pepmap C18 column

Run 50 min

Mass spectrometer

LTQ-Orbitrap Discovery

(Thermo Fischer)



Acquisition method:
Nth dependent method,
full MS on Orbitrap analyser,
MS/MS for the four most abundant ions on LTQ analyser

Proteins Identification

Raw data → mz XML

Software : X! Tandem
install in local version with perl scripts
(http://pappso.inra.fr/bioinformatique.html)
Database : EST goat+sheep and proteins *Bos taurus+ Capra hircus*

Results in XML data

Java script
Filter based on log (**E value protein) <-8**
2 differents peptides
Exel results

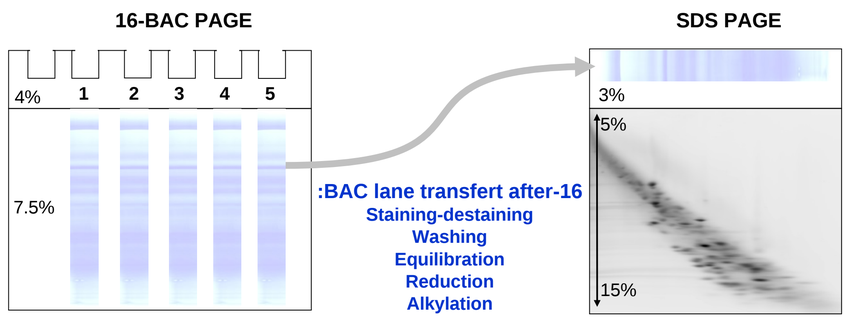
The GPM Web site
To visualize XML results
(http://www.thegpm.org/tandem/thegpm_upview.html

Unmatched mass spectra

PepNovo
http://cseweb.ucsd.edu/groups/bioinformatics/software.html
MSMS spectra quality<0.1
Score sequence>80 selected
Pepnovo data

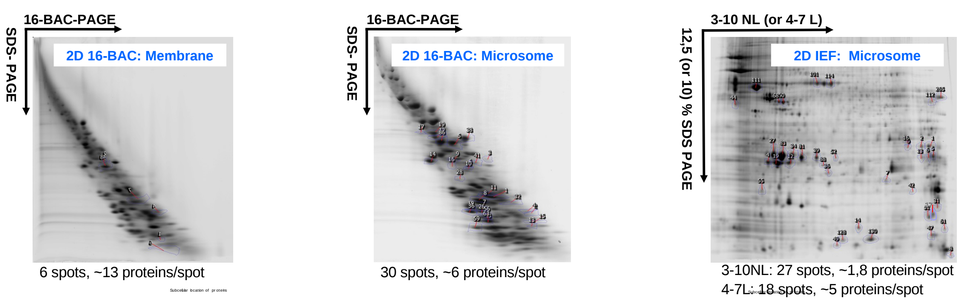
homology search with **Fasts** software (EBI) using the MD20-MS matrix
2 independants peptides and E value <0.001 were selected

Example of 2D DIGE experiment: 16-BAC/SDS PAGE

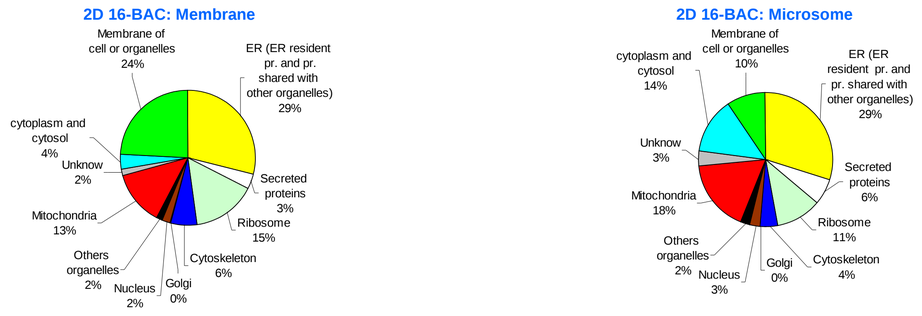


Results

2D DIGE gels: A/A *Vs.* O/O



Protein subcellular localization

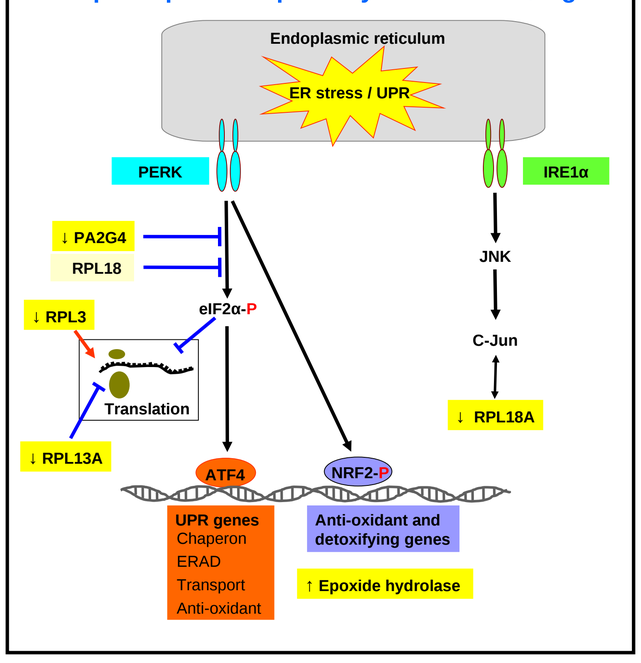


Proteins differentially expressed in O/O goat

	Upregulated in O/O goat	Down regulated in O/O goats
ER stress / UPR	1	1
Oxidative Stress	1	3
Translation		5
PTM	3	3
Transport	1	
Apoptosis, cell cycle	1	
Cytoskeleton	3	
Milk components	3	Apha S1 casein
Unknown function		1

The expression level of 12/27 proteins were validated by transcriptomic or western blot in defective goat, or the same result was shown in other pertinent studies

Example of pertinent pathway affected in O/O goat



Conclusions

- ✓ These proteomic results confirm previous findings (transcriptomic and RT-PCR of XBP1) showing that UPR is activated in mammary gland of defective goat
- ✓ The activation of UPR might explain, at least partially, the decrease of milk lipids in defective goat

Perspectives

- ➢ Additional key elements of UPR signaling pathway will be explored by Western blotting and qRT-PCR
- ➢ The genes of the UPR-induced apoptosis will be studied to evaluate the resistance of mammary epithelial cells to ER stress-induced apoptosis
- ➢ Proteomic results will be integrated with transcriptomic, biochemical and molecular results using the software *Ingenuity Pathway Analysis*

Our study will enhance our knowledge of the molecular mechanisms involved in milk protein and lipid secretions and might allow us to understand how the mammary tissue adapt to long-term UPR