

## LCM combined with MALDI-TOF Mass Spectrometry to demonstrate that $\alpha$ s1-casein is required for efficient export of the other caseins from the Endoplasmic Reticulum to the Golgi apparatus

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## LCM combined with MALDI-TOF Mass Spectrometry to demonstrate that $\alpha_{s1}$ -case in is required for efficient export of the other case ins from the **Endoplasmic Reticulum to the Golgi apparatus**

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**Casein micelles** 

Wild type: Homozygous A/A

RER

at the CSN1S1 locus

#### Background

Caseins, the main milk proteins, aggregate in the secretory pathway of mammary epithelial cells (MEC) into large supramolecular structures, the socalled casein micelles.

The role of individual casein in this process and the detail structure of the casein micelle remain poorly understood. In the goat species, a deficit in  $\alpha_{s1}$ casein is responsible for the accumulation of caseins in distended RER cisternae and for a perturbation of the secretion process of proteins and lipids that are synthesized as triglycerides at the ER level. In contrast, no accumulation was recorded in MEC lacking  $\beta$ -casein expression.

#### What are the mechanisms involved in MEC dysfunction?

# Differential proteomic analysis

O/O vs. A/A milks using 2D-DIGE



## Laser Capture Microdissection coupled with MALDI-ToF MS

#### impact IR laser quality control Fluorescence labelling - IFF across the caps GRP 04 Bioanalyzer of milk proteins O/O: Cy3 (green) Resul < 5 ng A/A: Cy5 (red) acini selection RIN >7 Isocitrate O deshvdrogenase **RNA** Extraction $\alpha_{c1}$ -casein is only present in A/A milk Goats are of different genotypes at the CSN1S2 locus ne expression analysis (qPCR) comparison of extreme genotypes A/A vs O/O at the*CSN1S1* locus: 100 additional proteins 3000 CEM in O/O milks (green spots, A/A some of which characterized in MS) 0/0 Direct analysis of sorteo ER-Resident proteins (GRP94, BiP, PDI): signing a singular secretory mechanism No $\alpha_{s1}\text{-}casein$ transcripts in the mammary tissue of « O/O » goats IECs in MALDI-Tof-MS 11305.03 **Proteomic imaging:** Intensi microdissected cells from « extreme β-CN\*A genotypes » at the CSN1S1 locus α-lac β-lg were compared. 18168.91 α<sub>s2</sub>-CN K-CN Caseins are detected only in O/O MS cells and are immature (not 24651.38 phosphorylated) meaning that they have not yet reached the Golgi 10,00 20,00 Conclusions Native Mass Materials accumulating in the ER of MEC from goats homozygous O/O at the CSN1S1 locus are immature milk proteins (without post-translational modifications). Our data strongly suggest that $\alpha_{s1}$ -casein



interacts with the other caseins in the rough endoplasmic reticulum to form a complex that is required for their efficient export to the Golgi apparatus where they will be phosphorylated to interact with Ca<sup>2+</sup> ions.

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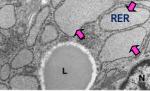


Micro

genomics

**Defective:** 

2014



Swelling of the Rough Endoplasmic Reticulum