GOAT MILK FAT GLOBULE MEMBRANE PHOSPHOPROTEINS

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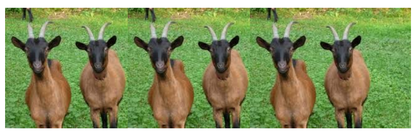
INTRODUCTION

Lipids are secreted into milk as droplets of triacylglycerols surrounded by a complex membrane originating from the mammary epithelial cell (MEC) and called the "Milk Fat Globule Membrane" (MFGM). Despite a consensus on broad outlines of lipid secretory pathways in the MEC, the identity of molecular partners engaged in these pathways is a controversial issue and several models for fat globules synthesis have already been reported. Analyzing the fine protein composition of the MFGM, the triple-layered membrane surrounding milk lipid droplets can provide mechanistic clues to better understand lipid droplet biosynthesis and secretion pathways in MEC. To improve the comprehension of this mechanism, we performed for the first time a phosphoproteome of an MFGM sample . To identify protein in the goat species, we combined a Q-Exactive LC-MS/MS analysis and because of incomplete database from goat, the creation of an in-house database. We used in house made software such as X!TandemPipeline (http://pappso.inra.fr/bioinfo/xtandempipeline) to group phosphopeptides by phosphosites and export results to the public database PROTICdb (Ref 1, http://pappso.inra.fr/protic/ptmgoat). In this last one, we implemented Olsen-Mann algorithm (phoscal) to predict the phosphorylation site(s).

METHODS

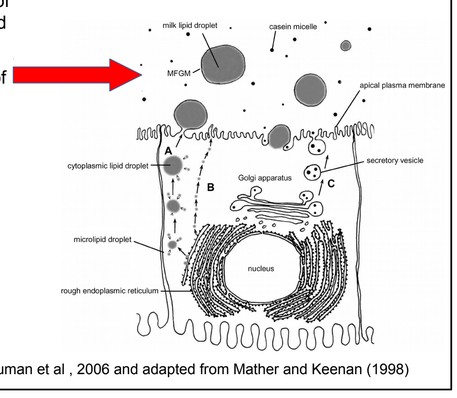
MFGM preparation



Milk sample from 6 six A/A genotype goats

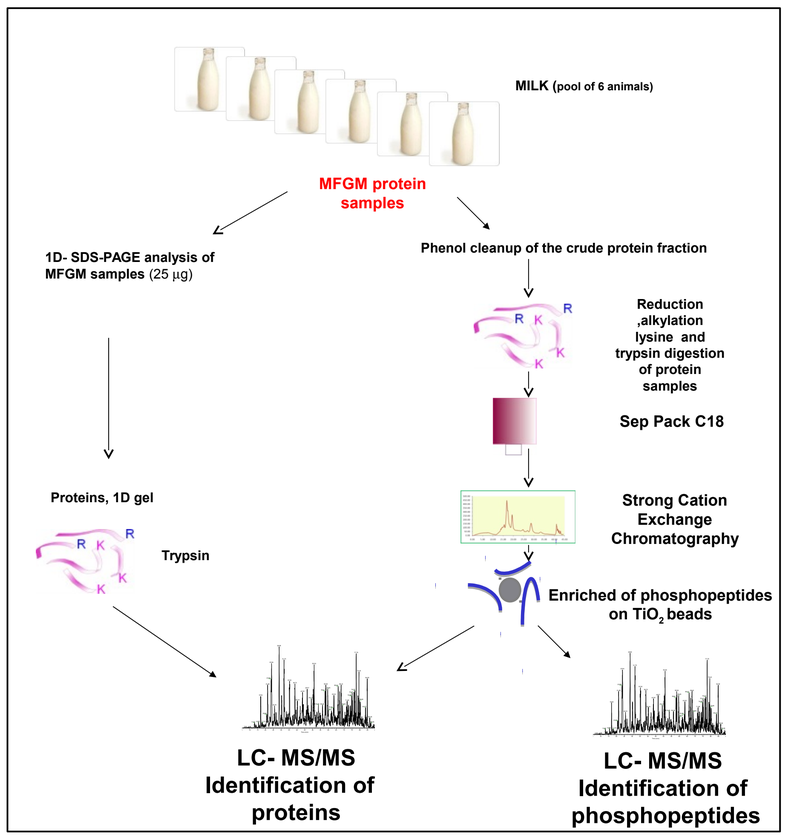
Caseins, which represent up to 80% of milk proteins, were efficiently removed from our MFGM preparations

MFGM : proteins represent only 1% of total milk proteins.



Major secretory pathways in mammary epithelial cells during lactation.
A = cytoplasmic lipid droplet pathway;
B = microlipid droplet pathway;
C = secretory pathway for skim milk components.
MFGM : Milk Fat Globul Membrane

Bauman et al , 2006 and adapted from Mather and Keenan (1998)



Identification of phosphopeptides

Injection of samples into nano HPLC coupled with Q- Exactive (Thermo Fisher Scientific)

Identification of phosphoproteins
X!Tandempipeline search engine (http://pappso.inra.fr/bioinfo/)
A combined of three databases :
Capra from UniprotKB (5352 entries)
Bovin from UniprotKB (38 794 entries)
Goat genedatabase (22 175 entries)

Localization probability : phoscal is implemented in PROTICdb

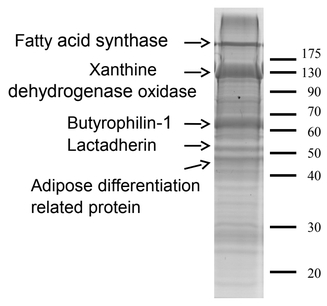
http://pappso.inra.fr/protic/ptmgoat

RESULTS

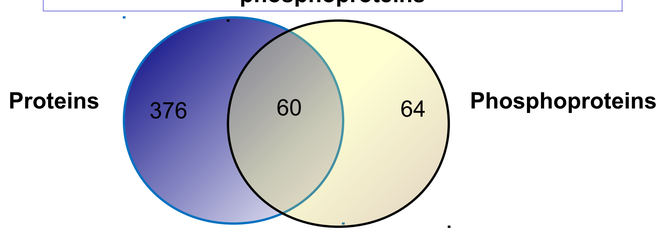
Identification of MFGM proteins

1D LC-MS/MS allowed us to identify 442 functionnal groups of proteins and 548 proteins.
More than XX % of MFGM proteins from goat were found to be integral membrane proteins (mostly belonging to the plasma membrane), or proteins associated to the membrane. Among them the three main proteins involved in model of secretion of lipid from MFGM: Butyrophilin, Xanthine dehydrogénase/oxydase, Lactadherin, Fatty acid synthase).

Enriched GO terms associated with MFGM proteins from goat milk were done with DAVID version 6,7 : protein translation (p-value = 1.10 × 10−19), vesicle mediated transport (p-value = 3.,23 × 10−12), glycolysis (p-value = 5.57 × 10−7), actin cytoskeleton organization (p-value = 2.11 × 10−05), and oxidation-reduction (p-value = 5.5 × 10−05).



Venn diagram of MFGM proteins and MFGM phosphoproteins

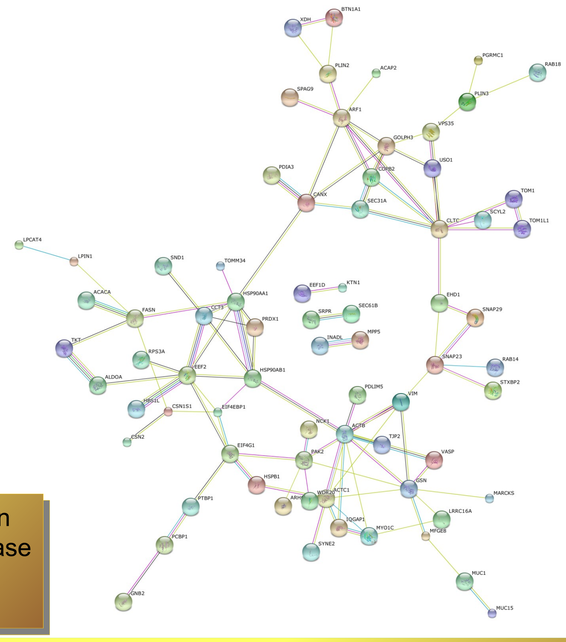


60 proteins were in common between the two datasets
Ratio of phosphopeptides S/T/Y : 211/ 52/ 8.

Identification of MFGM phosphoproteins

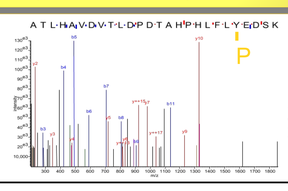
we identified 271 sites of phosphorylation on 124 unique MFGM phosphoproteins, among them several major protein display a high number of phosphosites.

Protein name (species)	Identified phosphorylation sites
Fatty acid synthase(Capra hircus)	**S58, S63, S207, S324, S427, S515, S519, S708, S713, S715, S724, T826, S830, T909, T974, T978, T1102, S1103, S1484, S1587, S1951, T1987, S2120, S2124, S2126, S2174, S2206, S2212, S2216, S2239, S2420, T2437, T2441**
Adipose differentiation-related protein (PLIN-2, Capra hircus)	**S59, S64, T120, T121, S129, T131, S132, T133, T135, S173, S174, S215, Y217, S222, S255, T256, S291, S293, S362, S428, S438, S443, S448, S449**
Butyrophilin subfamily 1 member A1 (BTN1A1, Capra hircus)	**S96, Y132, S157, T165, S166, S187, S189, S284, S285, T299, Y319, S343, Y359, T411, S492, S498, S504, S506, T511, S514**
Xanthine dehydrogenase oxidase (Capra hircus)	**T2, T25, S240, T382, Y393, T542, Y543, T544, S602, S620, S624, S711, T804, S871, S908, S964, S1299, T1302**
Milk fat globule-EGF factor 8 (MFG-E8 or lactadherin, Capra hircus)	**S193, S205**



Butyrophilin-1 which is essential to milk lipid droplets secretion and well known to be in interaction with Xanthine dehydrogenase Oxydase. 20 phosphosites were detected.

Example of MS2 mass spectra of Butyrophilin-1: Y319 phosphorylated



m/z= 995,805957
Z=3
X!Tandempipeline view

Interaction network (as displayed by EMBL STRING) of predicted associations for phosphorylated MFGM proteins.

The network nodes are proteins. The edges represent the predicted functional associations. Red line, fusion evidence; green line, neighborhood evidence; blue line, coocurrence evidence; purple line, experimental evidence; yellow line, text-mining evidence; light blue line, database evidence; black line, coexpression evidence. Disconnected nodes are hidden.

CONCLUSIONS

We performed the identification of 442 functional groups of proteins in the MFGM from goat milk.
271 sites of phosphorylation on 124 unique goat MFGM proteins were detected. Enriched GO terms associated with phosphorylated MFGM proteins were protein transport and actin cytoskeleton organization. The major proteins of MFGM, involved in milk lipid droplets secretion, were found with a very hight level of phosphorylation.
This results suggest phospho-dependent mechanisms regulating Lipid droplet trafficking or secretion from the mammary epithelial cells.
This will help the design of future studies regarding the role of reported phosphorylation sites in MFGM associated proteins in lipid droplets secretion from the mammary epithelial cells.

Ref 1: **Langella, O., Valot, B.,** Jacob, D., **Balliau, T.,** Flores, R., Hoogland, C., Joets, J. , **Zivy, M.** (2013) Management and dissemination of MS proteomic data with PROTICdb: Example of a quantitative comparison between methods of protein extraction. *Proteomic*, pmic.201200564
Ref 2: **Henry,C.** Saadaoui,B., Bouvier,F., Cebo,C., (2015) ;Phosphoproteomics of the goat milk fat globule membrane: New insights into lipid droplet secretion from the mammary epithelial cell *Proteomic*, pmic.201400245