

#### GOAT MILK FAT GLOBULE MEMBRANE PHOSPHOPROTEINS

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# **GOAT MILK FAT GLOBULE MEMBRANE PHOSPHOPROTEINS**

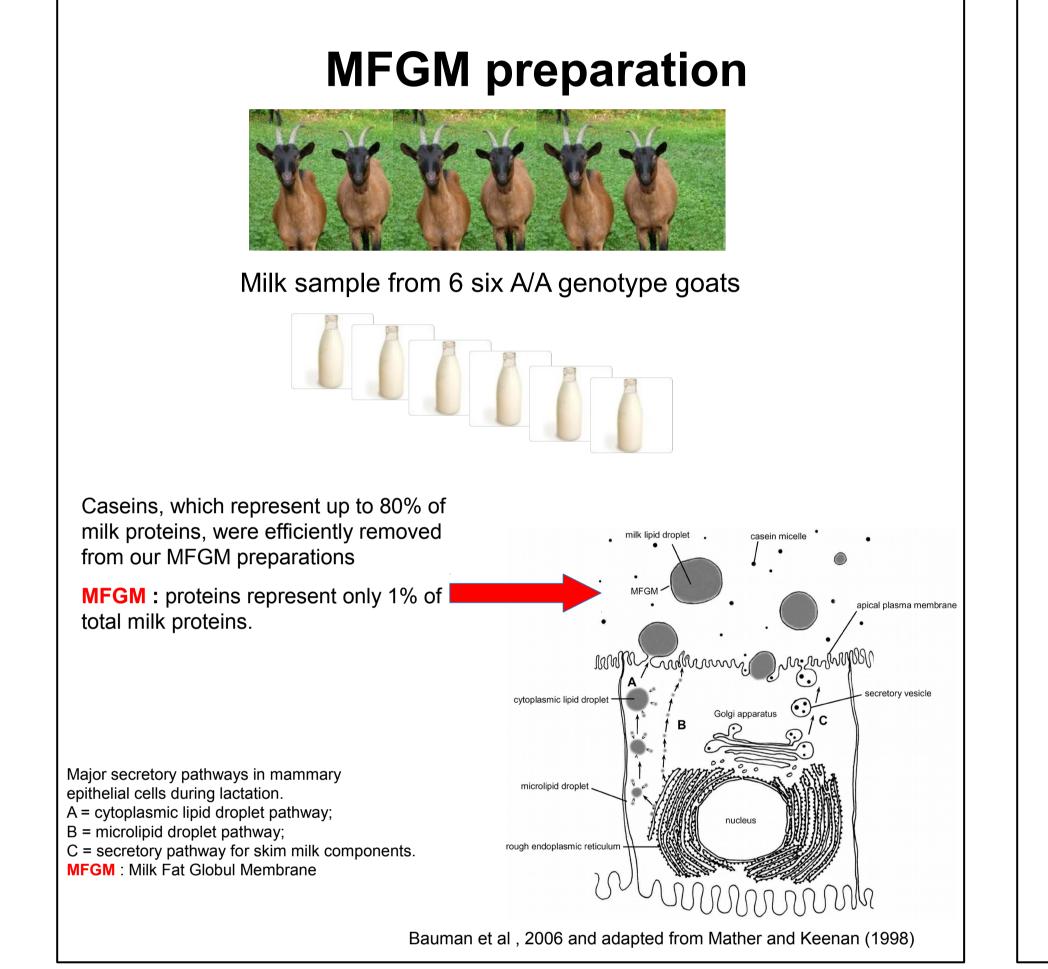
### Céline Henry<sup>1</sup>, Besma Saadaoui<sup>2</sup>, Frederic Bouvier<sup>3</sup>, and Christelle Cebo<sup>4</sup>

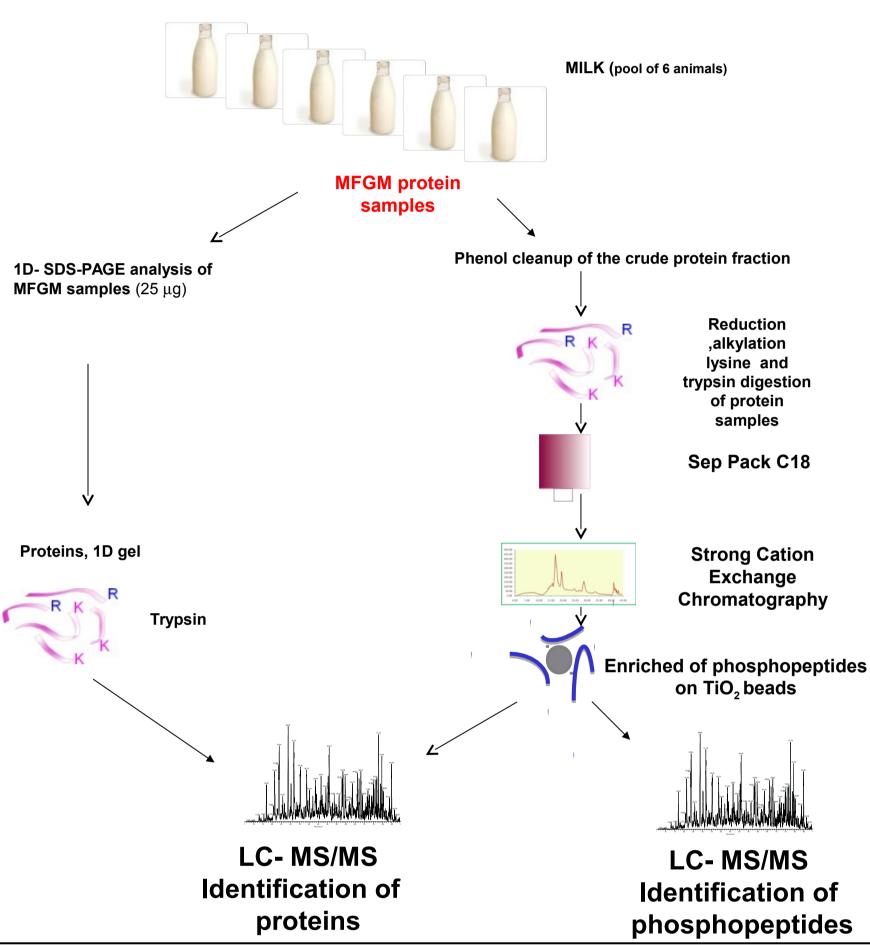
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## INTRODUCTION

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 export results to the public database PROTICdb (http://pappso.inra.fr/bioinfo/xtandempipeline) to group phosphopeptides by phosphosites and (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**





RESULTS

Fatty acid synthase —>

dehydrogenase oxidase

Lactadherin

Adipose differentiation

related protein

Xanthine

Butyrophilin-1 —>

#### 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

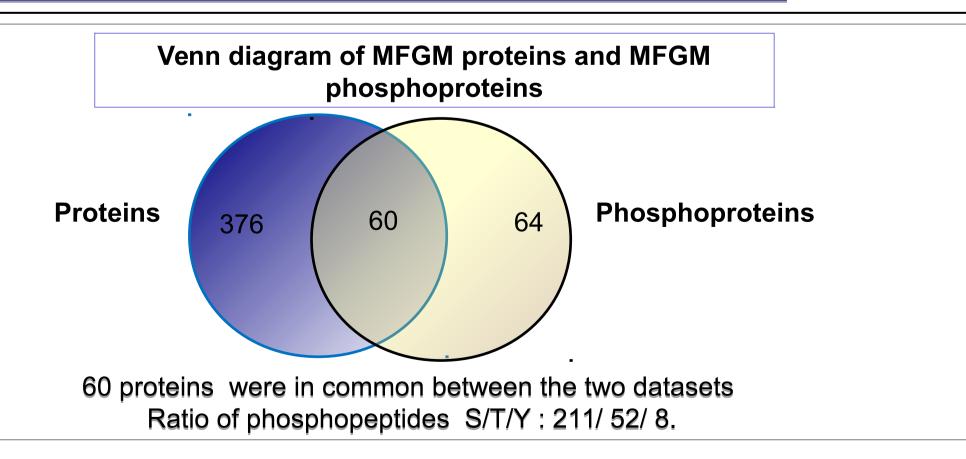
http://pappso.inra.fr/protic/ptmgoat

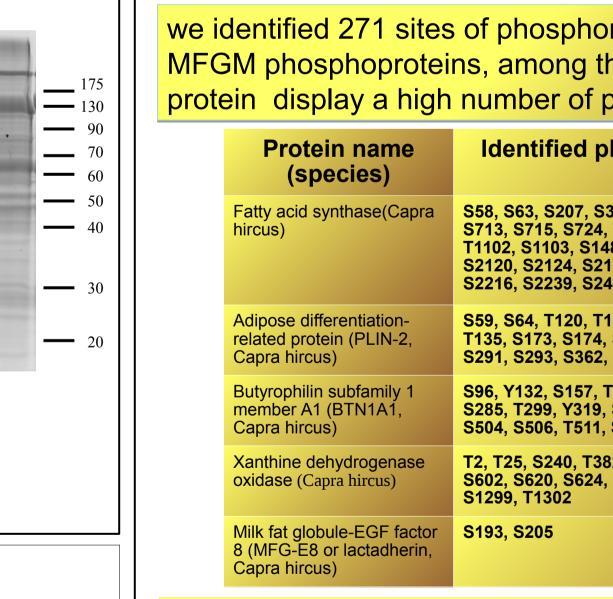
#### Identification of MFGM proteins

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).

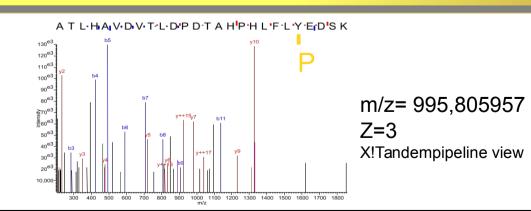
Enriched GO terms associated with MFGM proteins from goat milk were done with DAVID version 6,7 : protein translation (p-value = 1.10)  $\times$  10–19), vesicle mediated transport (p-value = 3.,23  $\times$  10–12), glycolysis (p-value =  $5.57 \times 10-7$ ), actin cytoskeleton organization (pvalue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





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.

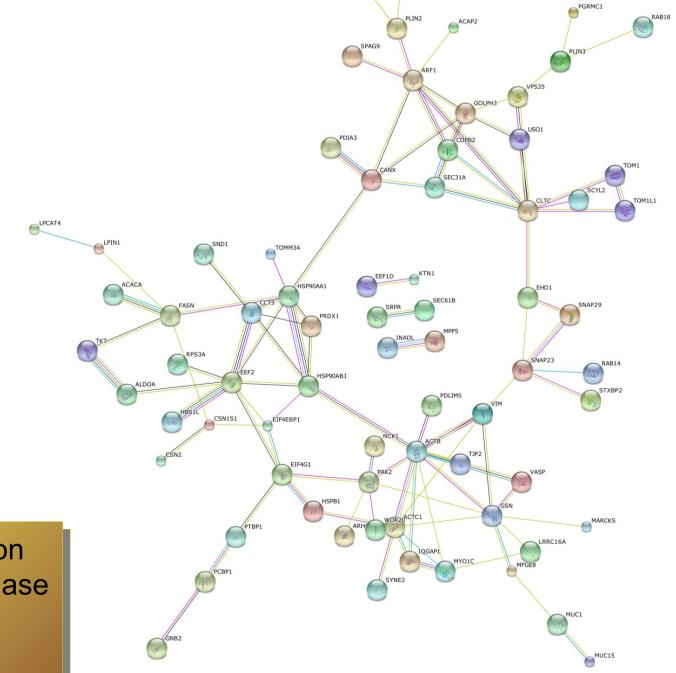
Example of MS2 mass spectra of Butyrophilin-1: Y319 phosphorylated



## 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
acid synthase(Capra s)	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
ose differentiation- ed protein (PLIN-2, a 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
ophilin subfamily 1 ber A1 (BTN1A1, a hircus)	<mark>S96, Y132, S157, T165, S166, S187, S189, S284, S285, T299, Y319, S343, Y359, T411, S492, S498, S504, S506, T511, S514</mark>
nine dehydrogenase se (Capra hircus)	T2, T25, S240, T382, Y393, T542, Y543, T544, S602, S620, S624, S711, T804, S871, S908, S964, S1299, T1302
at globule-EGF factor G-E8 or lactadherin, a hircus)	S193, S205
hilip 1 which	is essential to milk linid dronlets



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.



#### 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