

SMAP 2009

**Spectrométrie de Masse
et Analyse Protéomique**

DIJON

Congrès de la Société
Française d'Electrophorèse
et d'Analyse Protéomique

26^{èmes} Journées
Françaises de la
Spectrométrie de Masse



Soumission de resume

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

Lahouassa Hichem (1), Henry Céline (2), Beauvallet Christian (3), Badaoui Bouabid (1), Martin Patrice (4), Chanat Eric (5)

(1)INRA, UR1196 Génomique et Physiologie de la Lactation, F-78352 Jouy-en-Josas France.
(2)INRA, UR 477, Biochimie bactérienne, plateforme d'analyse protéomique de Paris Sud Ouest (PAPPSO), F-78352 Jouy-en-Josas FRANCE. (3)INRA, UMR1313, Génétique animale & Biologie intégrative, équipe « LGS-PiCTGem », F-78352 Jouy-en-Josas FRANCE. (4)INRA, UMR1313, Génétique animale & Biologie intégrative, équipe « LGS-PiCTGem », F-78352 Jouy-en-Josas France. (5)INRA, UR1196 Génomique et Physiologie de la Lactation, F-78352 Jouy-en-Josas France.

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.

It has been demonstrated that the lack of CSN1S1 in goat homozygous for the null allele (O/O) induces the accumulation of the other caseins in the ER, a phenomenon which, in turn, causes an Unfolded Protein Response (UPR). In addition, the secretion of both milk proteins and lipids in the milk of defective goat was shown to be dramatically affected. Links between these two secretory processes are therefore very likely.

To better understand the molecular mechanisms driving these two essential secretory functions, a differential proteomic analysis of goat mammary rough endoplasmic reticulum of extreme CSN1S1 genotypes (A/A vs. O/O) was undertaken.

Rough endoplasmic reticulum microsomes (RERM) were purified essentially as described by Paiement et al. High quality purification was confirmed by electron microscopy and biochemical analysis.

Whole RERM or its purified membranes were analyzed by 2D difference gel electrophoresis (DIGE), IEF/SDS-PAGE and/or 16-BAC/SDS-PAGE. Conventional 2D (IEF/SDS-PAGE) is the main technique used for soluble proteins. However separation of strongly acidic, basic or membrane proteins is still limited. To cope with this problem we have used a gel electrophoresis in an acidic buffer system using the cationic detergent (16-BAC) associated with SDS-PAGE. Subsequently, characterization of statistical relevant proteins was performed by LTQ-Orbitrap, (Thermo Electron). Because of the goat genome is non available yet, we have used in coupled Xtandem (<http://www.thegpm.org/TANDEM/>) and PepNovo (<http://cseweb.ucsd.edu/groups/bioinformatics/software.html>) software.

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Most identified proteins were associated with pertinent pathways: ER stress, including oxidative stress, and the UPR, membrane traffic, protein translation and apoptosis. The activation or inhibition of some pathways were clearly confirmed by Western blot analysis of relevant proteins.

Our study demonstrated that, in defective goat, mammary epithelial cells adapt to resist to the chronic ER stress and to avoid apoptosis. This study also improves our knowledge about the molecular mechanisms involved in milk protein and lipid secretions.

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Informations sur l'auteur

NOM : LAHOUASSA

PRENOM : HICHEM

EMPLOYEUR : INRA, UR1196 GÉNOMIQUE ET PHYSIOLOGIE DE LA LACTATION

ADRESSE

DOMAINE DE VILVERT

F-78352 JOUY-EN-JOSAS

FRANCE

TELEPHONE : 0134652548

EMAIL : hlahouassa@jouy.inra.fr

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