# *Effect of a 48h food deprivation on the lactating goat mammary miRNome*

**Lenha Mobuchon[1, 2]**, Sylvain Marthey[1], Sandrine Le Guillou[1], Denis Laloë[1], Fabienne Le Provost[1] and Christine Leroux[2]
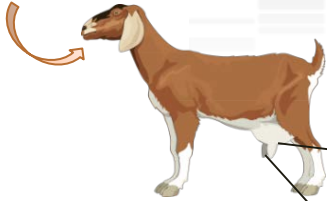
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**[1]UMR 1313 Animal Genetics and Integrative Biology, F-78350 Jouy-en-Josas, France**
**[2]UMR 1213 Ruminants, F-63122 Theix, France**
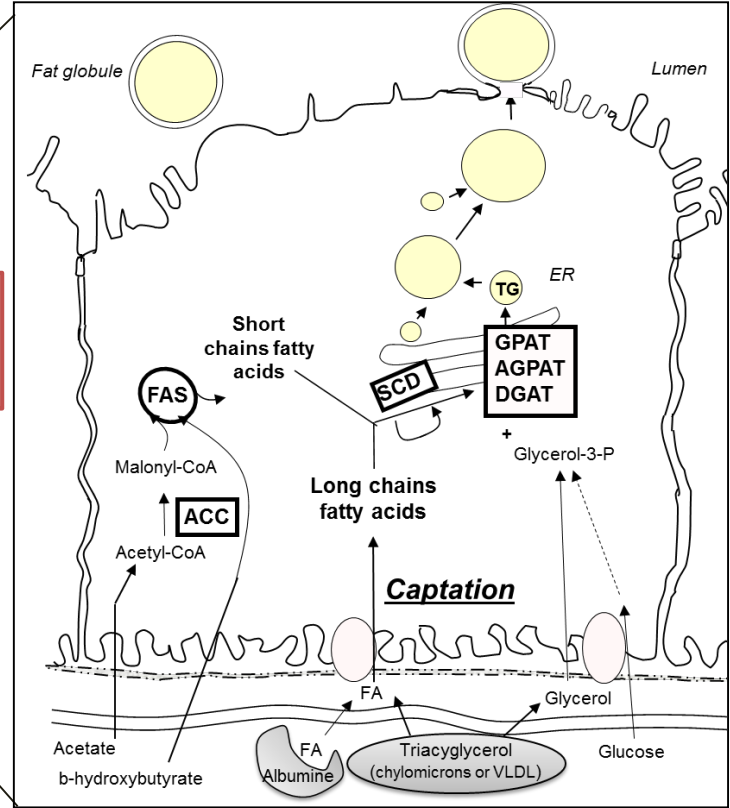
# Mammary Nutrigenomic

*Nutrition*



? 

Change in genes expression

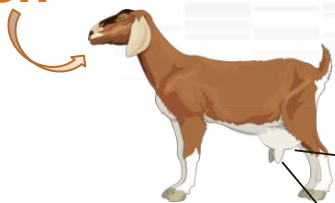Change in milk composition
(fat, proteins and lactose)

**Milk:** Fat (35g/L)
Nutritional value for the consumer.

MILK

[Bernard et al., 2008]

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# Mammary Nutrigenomic

*Nutrition*

48h

**161 Differentially Expressed Genes (DEG)**
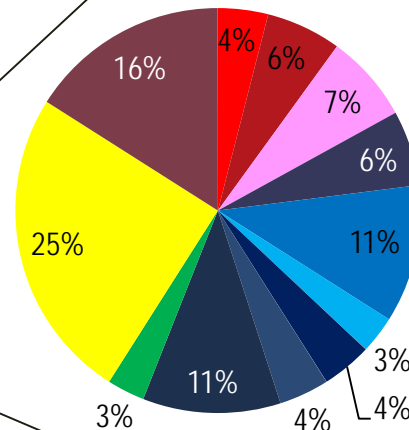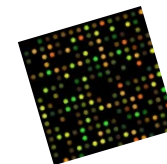
?

■ Cell adhesion and cytosquette
■ Signal transduction
■ Cell life
■ Transcription and RNA metabolism
■ Metabolism and transport of proteins
■ Major milk proteins
■ Metabolism and transport of lipids
■ Various metabolism
■ Various transport
■ Others
■ Biological processes unknown
■ EST

Pie chart values: 4%, 6%, 7%, 6%, 11%, 3%, 4%, 4%, 11%, 3%, 25%, 16%

↘ **Milk yield**
↘ **Lactose, Protein & Fat**
**Change in fatty acids composition**

**Change in genes expression:**
**-5 major milk proteins + 3 lipogenic enzymes**

**Milk**

*Mechanism of regulation ?*

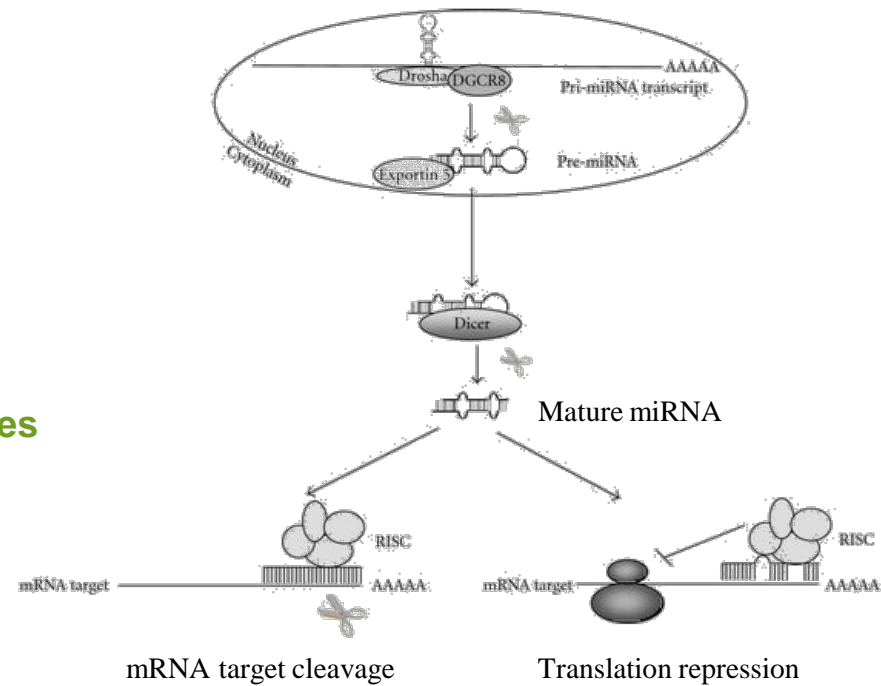*[Ollier et al., 2007]*

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# MicroRNA

➢ Small non coding RNA of 19-24 nt
➢ High conservation between species
➢ Mainly repress translation of their mRNA targets

**=> Involved in most of the biological processes**

➢ Regulation: Examples of the **nutriregulation** :

| miRNA | Tissue/species | Diet | *Referencies* |
|---|---|---|---|
| miR-122 | Liver/pig | High cholesterol | *Cirera et al., 2010* |
| miR-140-3p | Epiphyseal growth plate/rodents | Caloric restriction | *Pando et al., 2012* |
| miR-204-5p | Adipose tissue/mouse | High fat | *Chartoumpekis et al., 2012* |

Drosha DGCR8   Pri-miRNA transcript   AAAAA
Nucleus / Cytoplasm   Exportin 5   Pre-miRNA
Dicer
Mature miRNA

mRNA target   RISC   AAAAA
mRNA target cleavage

mRNA target   RISC   AAAAA
Translation repression

In the mammary gland ?

*Others examples …*
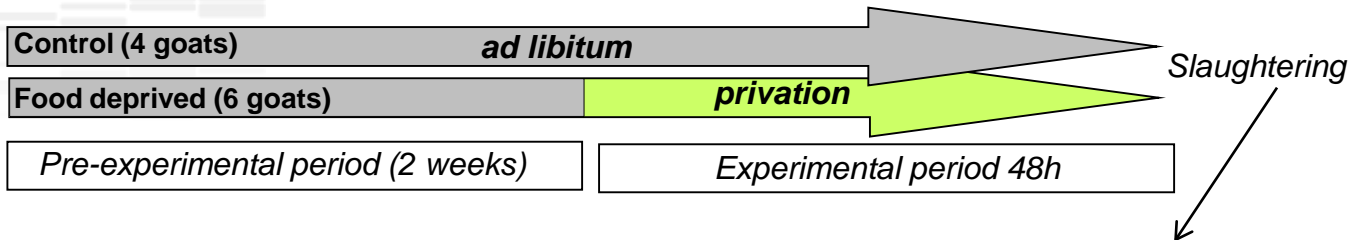
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# Objectives

Identification of **nutritional regulation** of miRNA expression in the goat mammary gland.

# Materials and Methods

**10**

Alpine goats
(48±2 days of lactation)

Control (4 goats) — *ad libitum*

Food deprived (6 goats) — *privation*

*Slaughtering*

Pre-experimental period (2 weeks)

Experimental period 48h

Sequencing data treatment:
- ✓ Remove sequencing adapters
- ✓ Size filter -> 17-28 nt in lenght
- ✓ Mapping, annotation and quantification:

**miRDeep2** software

Mammary gland sampling:
- ✓ RNA extraction
- ✓ Next Generation Sequencing (Illumina, Solexa)

Differential analysis:
**HTSFilter** and **DESeq2** R packages

Validation: RT-qPCR →

Target prediction:
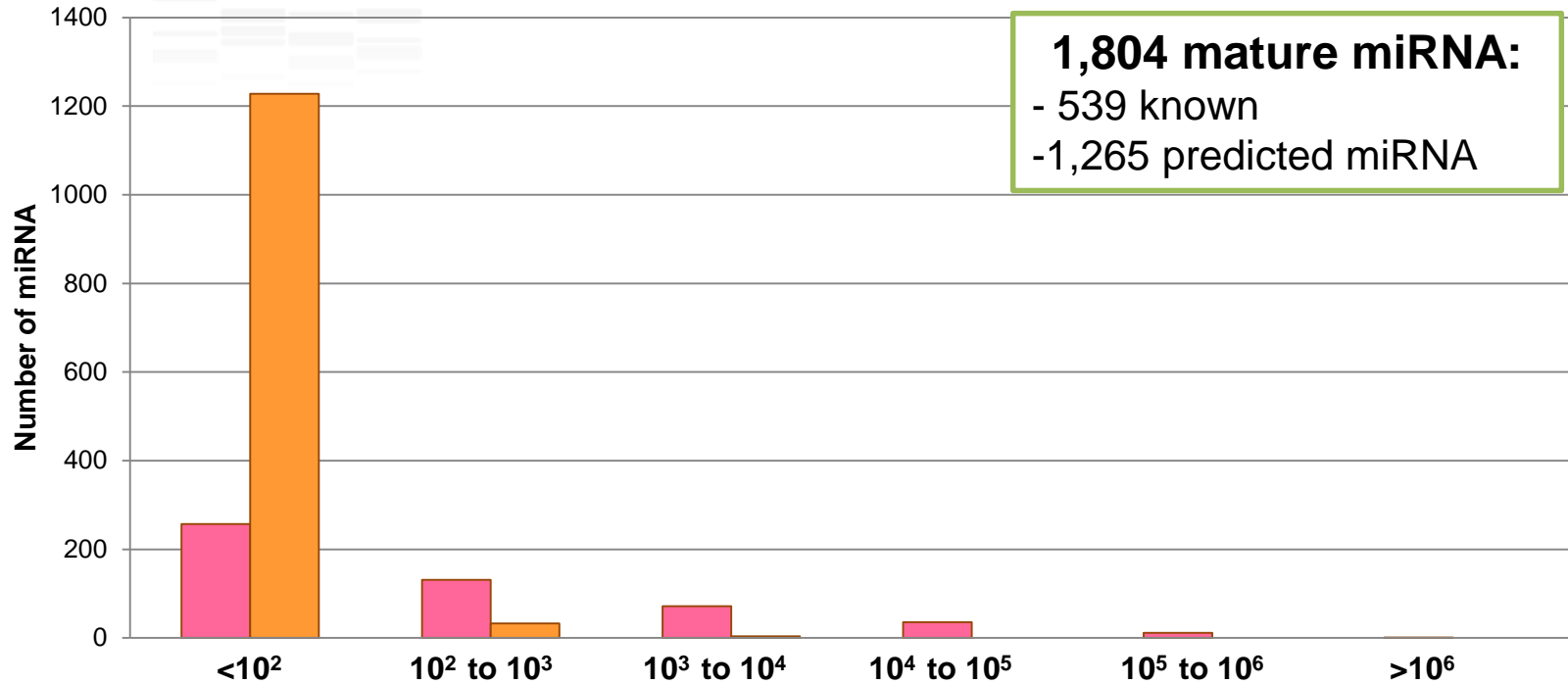Computational applications
(TargetScan, DIANA and IPA)

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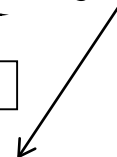
# Mammary miRNomes

Exhaustive list of miRNA expressed in a tissue

**1,804 mature miRNA:**
- 539 known
- 1,265 predicted miRNA



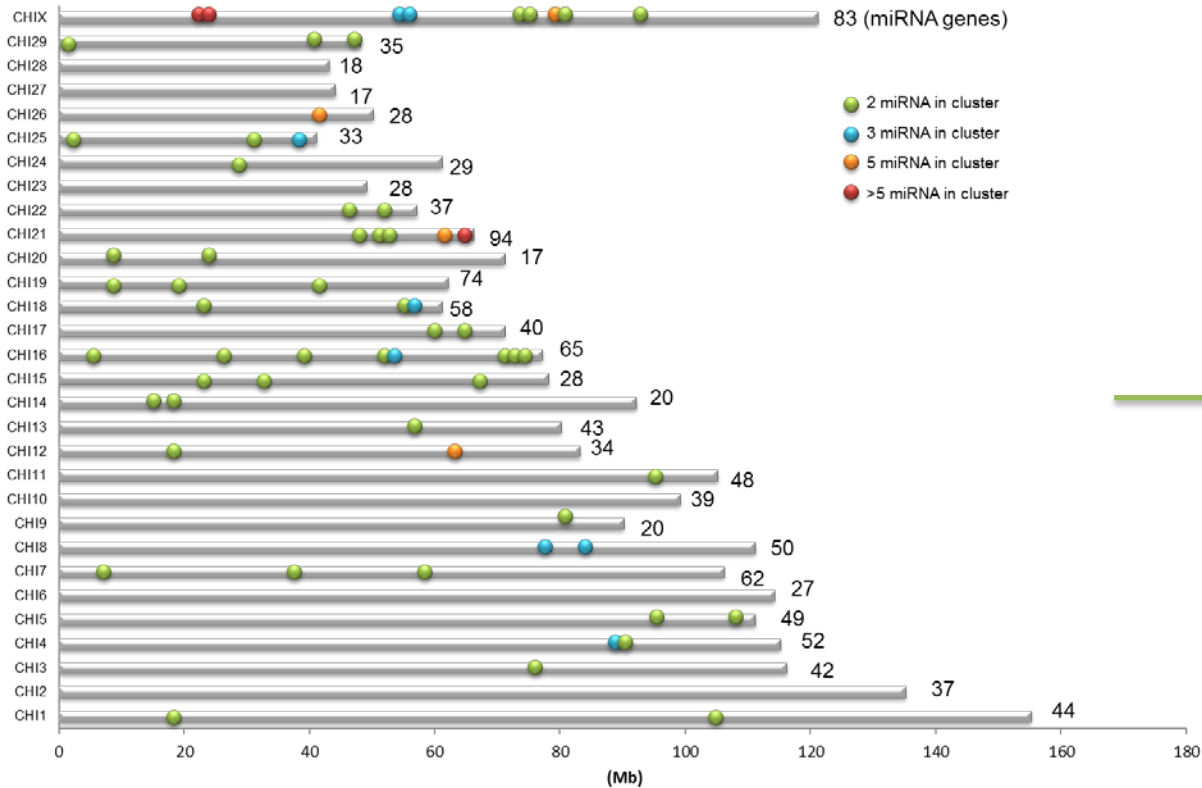Chart: Number of miRNA vs expression level categories ($<10^2$, $10^2$ to $10^3$, $10^3$ to $10^4$, $10^4$ to $10^5$, $10^5$ to $10^6$, $>10^6$)
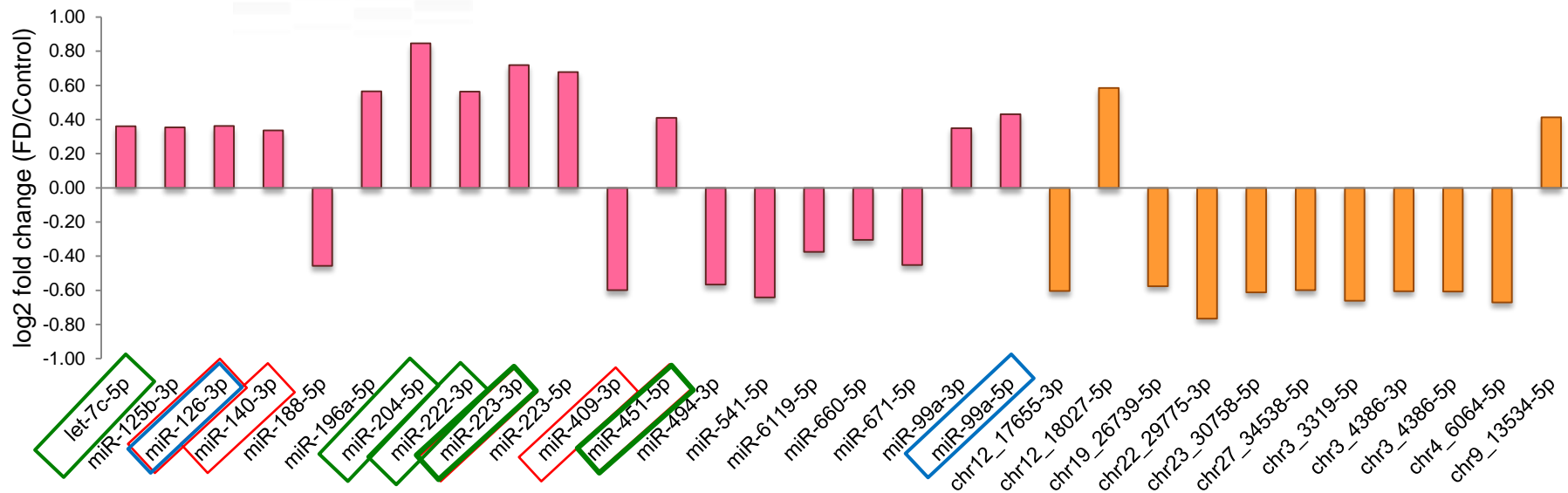
# Annotation of the caprine genome



Establishment of a reference map of goat miRNA on genome

*[Mobuchon et al., 2014, submitted]*

# Mammary nutriregulated miRNA

30 nutriregulated miRNA (n=6, p< 0,10), **19 known** and **11 predicted** miRNA



Already described in restriction challenge
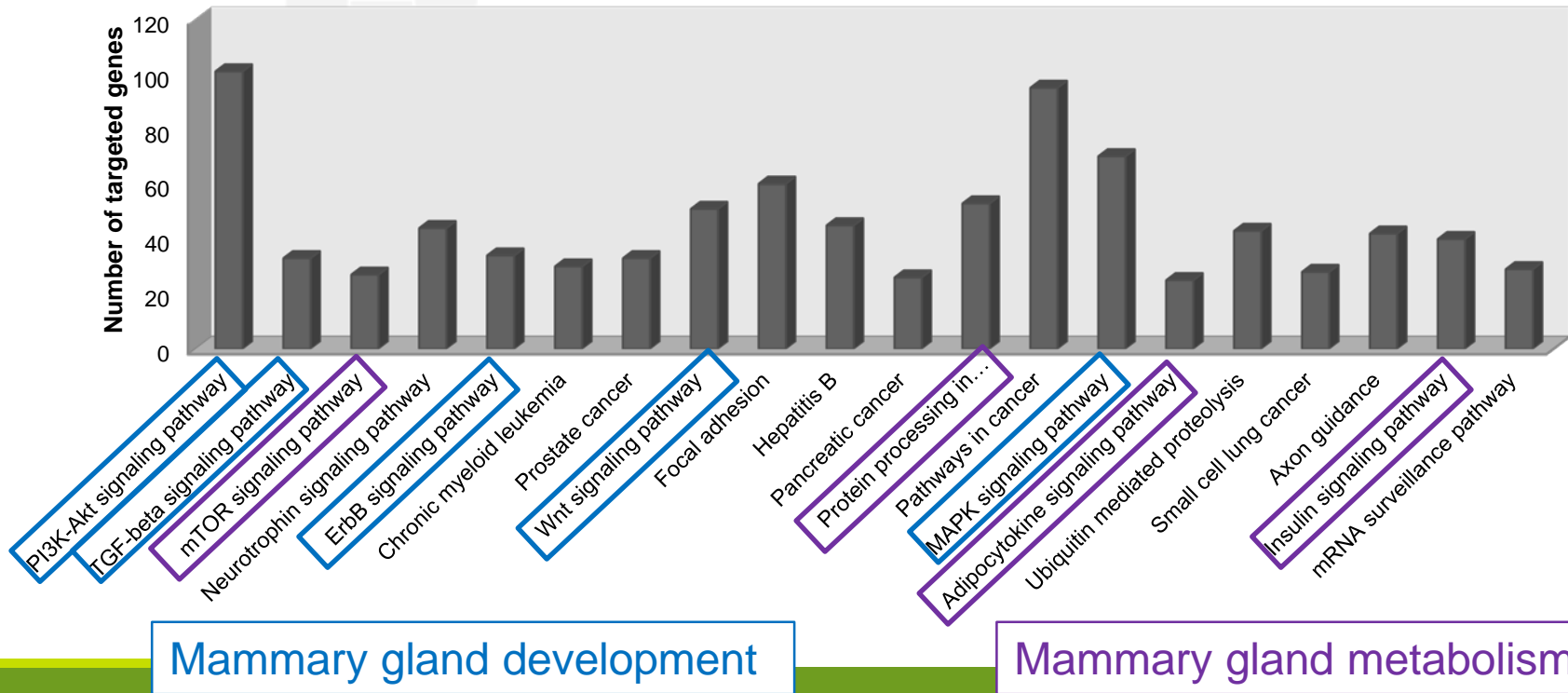*[Pando et al., 2013, Mercken et al., 2013, Dhabi et al., 2013]*

Involved in mammary epithelial cells proliferation
*[Cui et al., 2011,Turcatel et al.,2012]*

Described in lipid metabolism
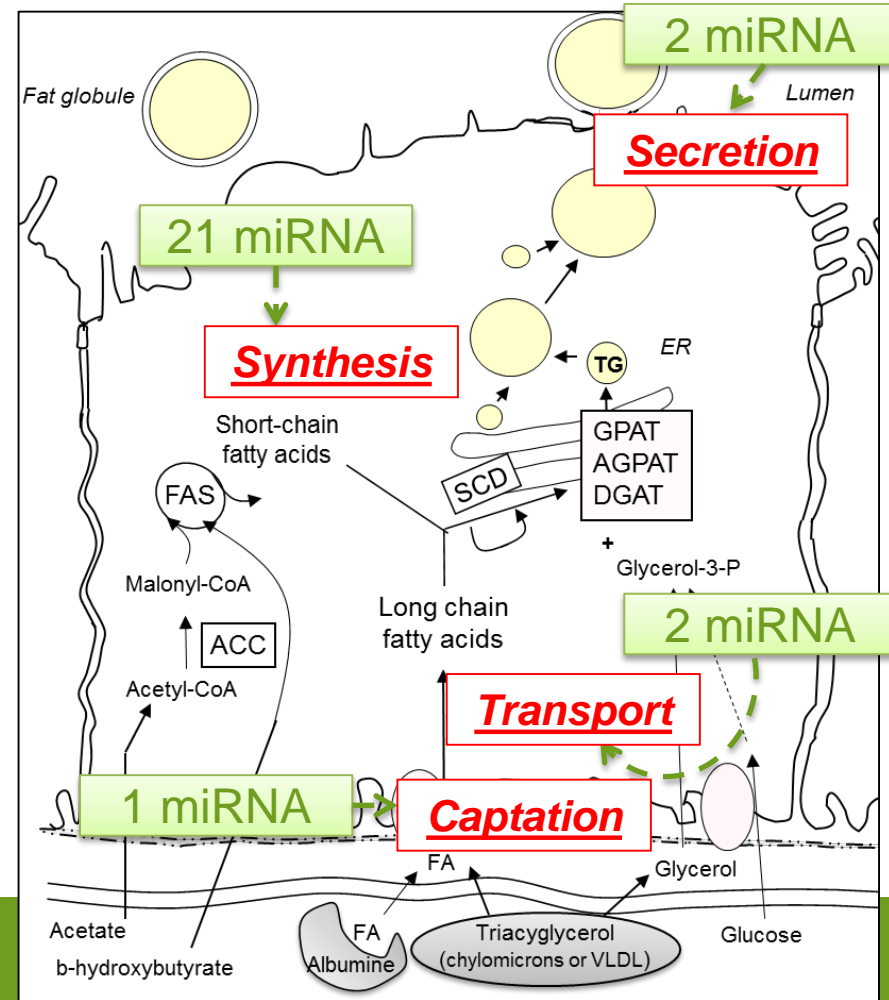*[Civelek et al., 2013, Hwang et al., 2013,Wagner et al, 2013]*

# Putative targeted pathways by nutriregulated miRNA



Mammary gland development
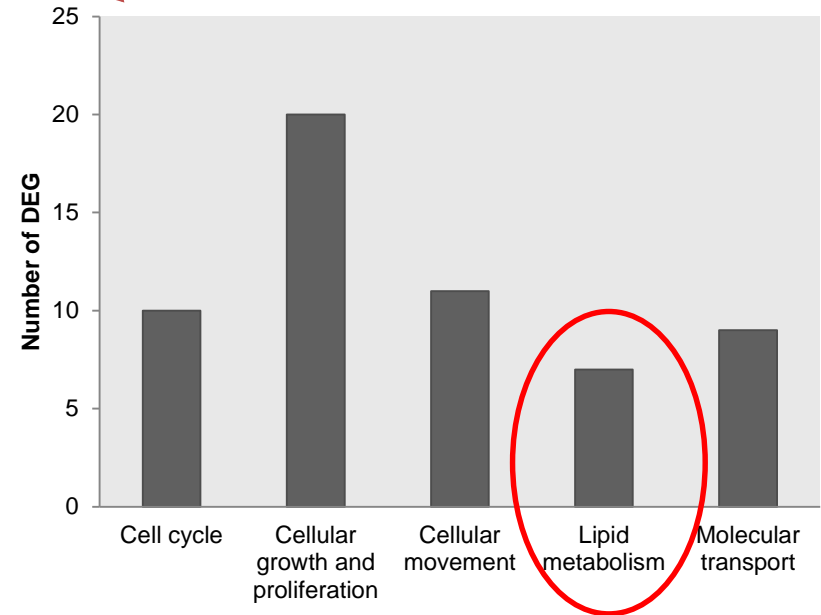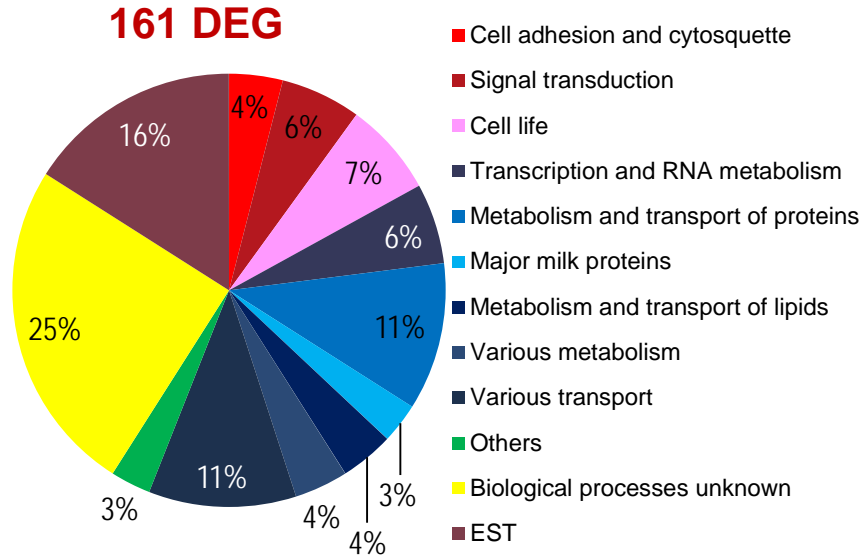
Mammary gland metabolism

# Putative pathways

➢ Many putative targets involved in mammary lipid metabolism

➢ Many mammary amino acids transporters, ex: SLC7A5, SLC38A2…

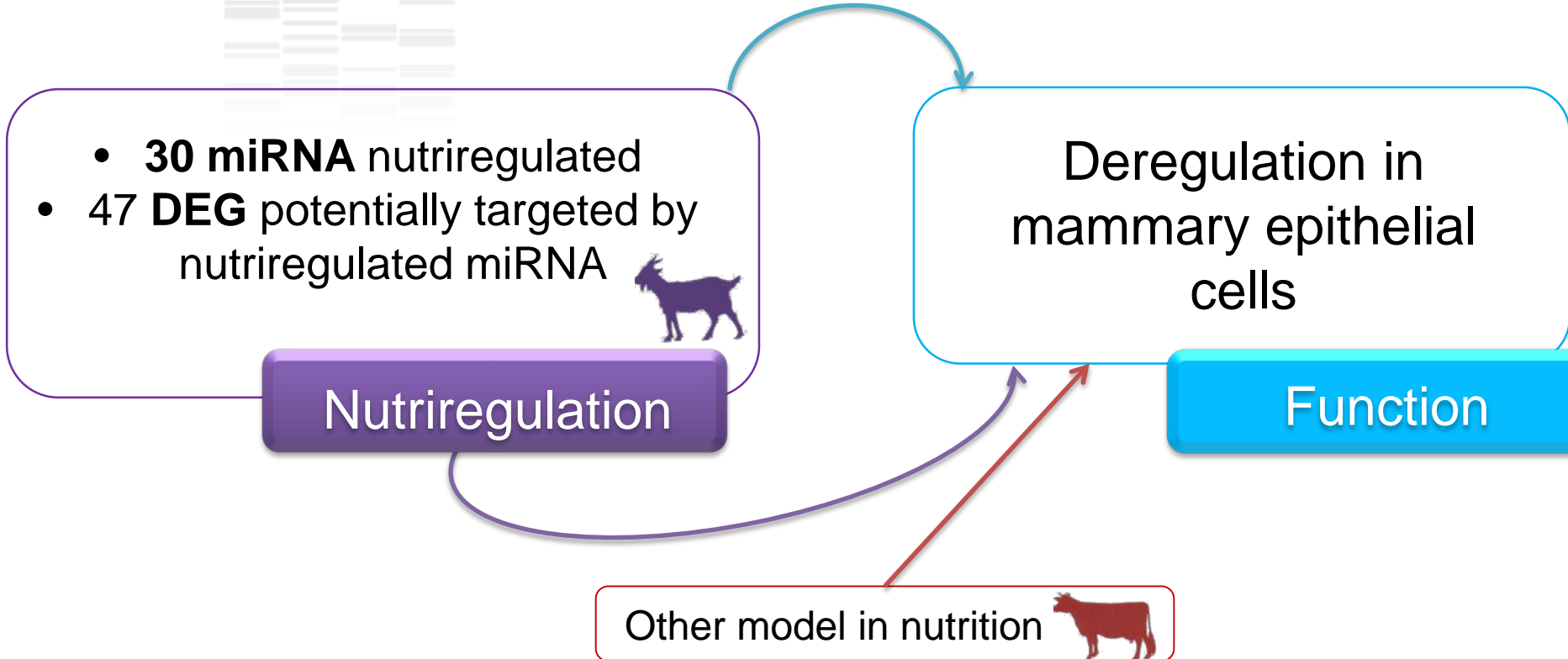**Involvement in milk product synthesis ?**

# Nutriregulated miRNA and DEG

**47 DEG** potentially targeted by nutriregulated miRNA



**161 DEG**

- Cell adhesion and cytosquette
- Signal transduction
- Cell life
- Transcription and RNA metabolism
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# Conclusion and perspectives

- **30 miRNA** nutriregulated
- 47 **DEG** potentially targeted by nutriregulated miRNA

**Nutriregulation**

Deregulation in mammary epithelial cells

**Function**

Other model in nutrition
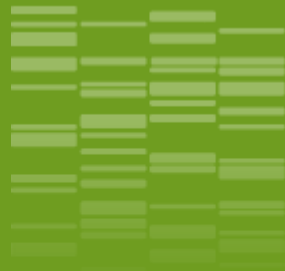
# Thank you for your attention