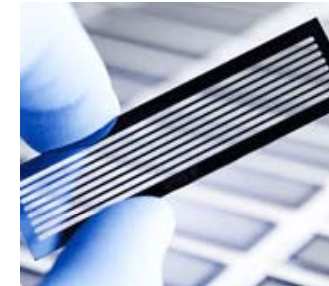




Analysis of bovine milk transcriptome using RNA-sequencing

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IMGIC, Davis, CA - 2010



Milk

- Milk is a complex bioactive fluid consumed by new born mammals
- Composition and physical characters of milk vary from species to species



Species	Water	Fat	Casein	Whey	Lactose	Ash
Human	87.1	4.6	0.4	0.7	6.8	0.2
Cow	87.3	4.4	2.8	0.6	4.6	0.7
Buffalo	82.2	7.8	3.2	0.6	4.9	0.8
Goat	86.7	4.5	2.6	0.6	4.4	0.8
Sheep	82	7.6	3.9	0.7	4.8	0.9
Reindeer	66.7	18	8.6	1.5	2.8	1.5

Composition of milk (g/100g)

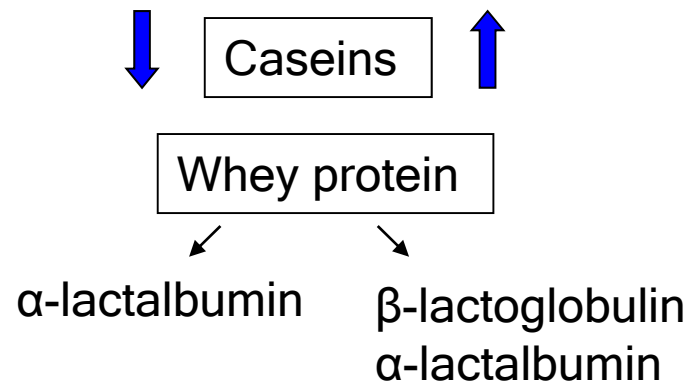


Cow milk

- Cow milk is consumed by humans beyond infancy

Species	Water	Fat	Casein	Whey	Lactose	Ash
Human	87.1	4.6	0.4	0.7	6.8	0.2
Cow	87.3	4.4	2.8	0.6	4.6	0.7

- There are differences between the cow milk and human milk





Characterization of cow milk

- Even though the chemical and physical properties of cow milk are well characterized, very limited research has been done on characterizing the milk transcriptome.

$$P = G + E + G * E$$

Phenotypic value = Biological properties of milk

Genotypic value = Controlled by many genes (quantitative trait)

Environment = Nutrition, climate, lactation stage, diseases

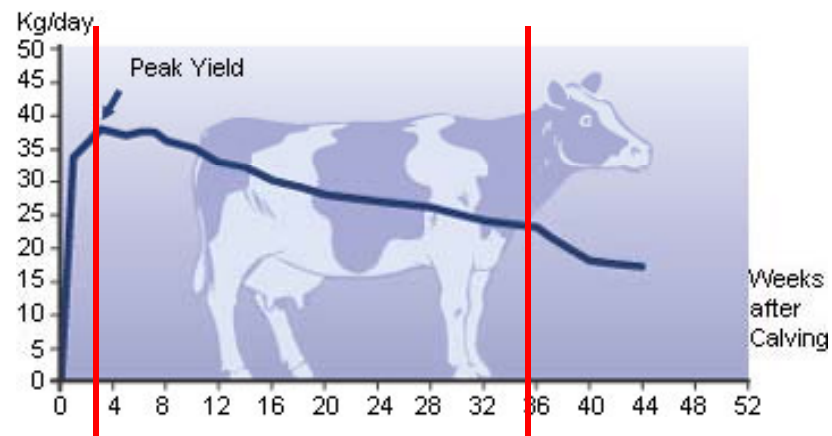


Objective

- To perform a comprehensive expression profiling of genes expressed in transition (day 15) and late (day 250) lactation milk cells in Holstein cows



Procedure



Three Holstein cows/2nd lactation
D15 (transition) and D250 (late) milk



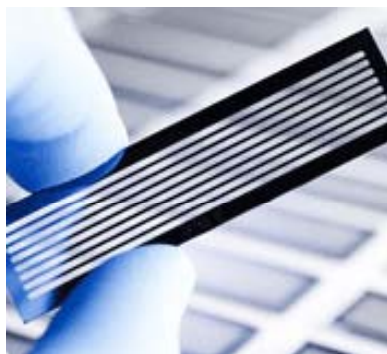
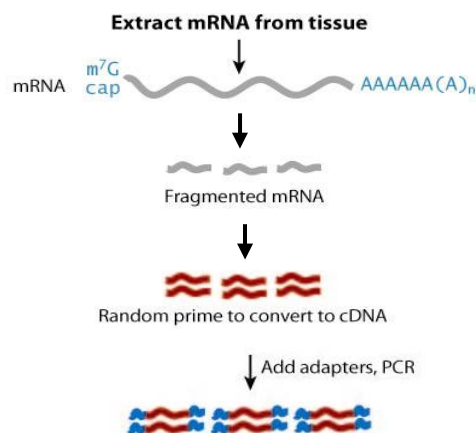
Extract RNA from milk cells



RNA sequencing



RNA sequencing (RNA-seq)



Illumina RNAseq

- Millions of reads, 36-40bp length
- Transcripts for 24,580 unique genes
- Expression range 0 - 493,430 RPKM

RPKM: Reads Per Kilobase per Million mapped reads



Analysis of data

CLC Genomics workbench 3.7



Map 40bp tags to reference bovine genome assembly
(Btau4.0-*Ensembl* annotation, 24,580 genes)



Normalization- RPKM



threshold for detectable gene expression = 0.3 RPKM



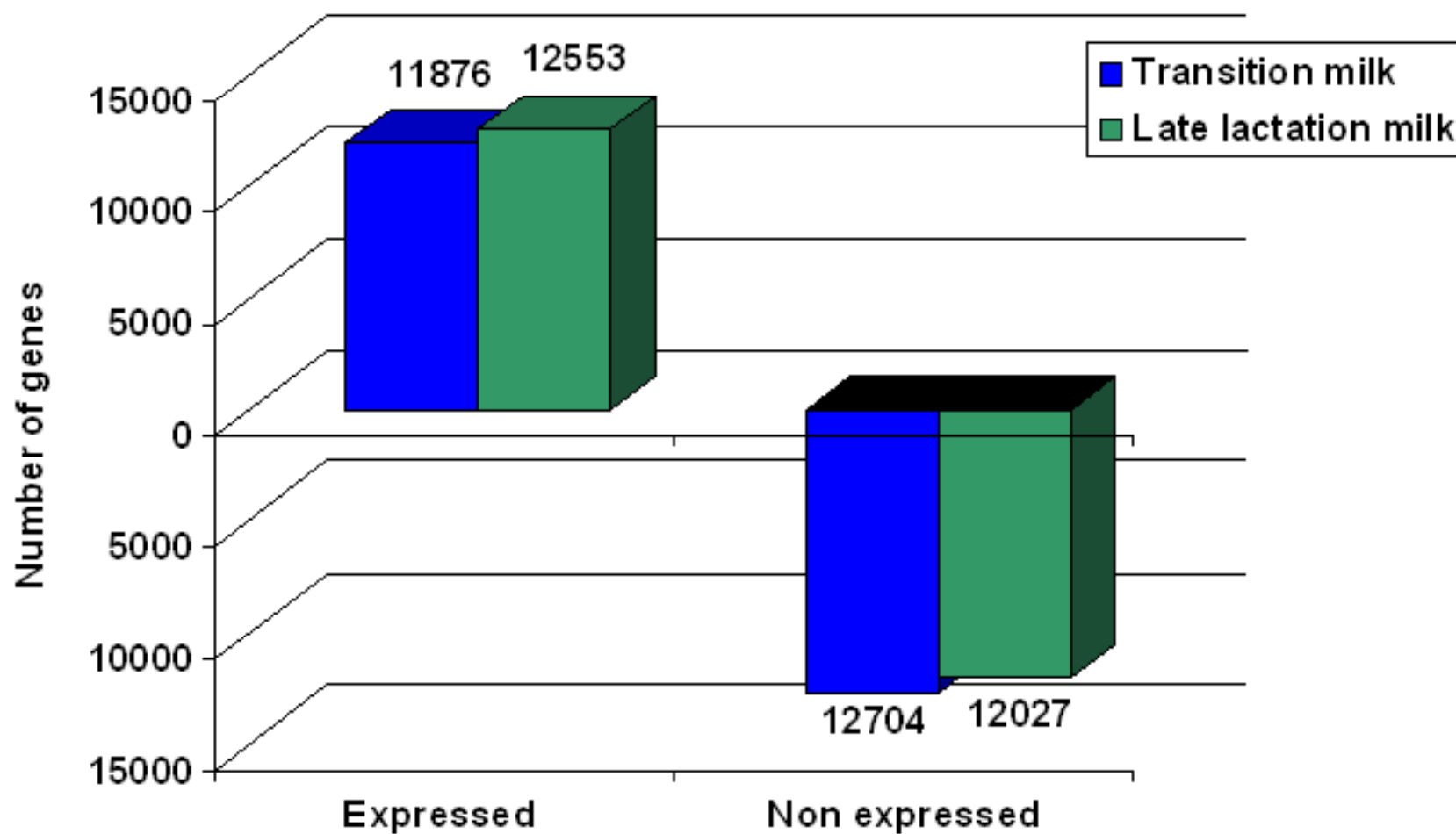
Statistical analysis - t-test
 $p \leq 0.05$, FDR $q \leq 0.3$



Significant GO terms
GenMAPP/MAPPFinder
Permutation $p \leq 0.05$



Results

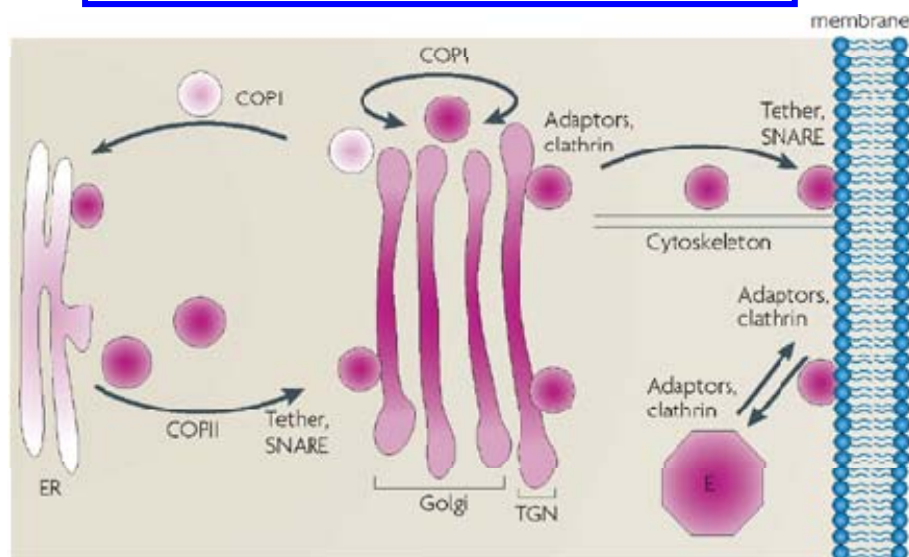


(Btau4.0-*Ensembl* annotation, 24,580 genes)



GO annotation results

Transition milk (day 15)



Nature Reviews | Molecular Cell Biology

Golgi vesicle and intra-Golgi transport
(Biological process)

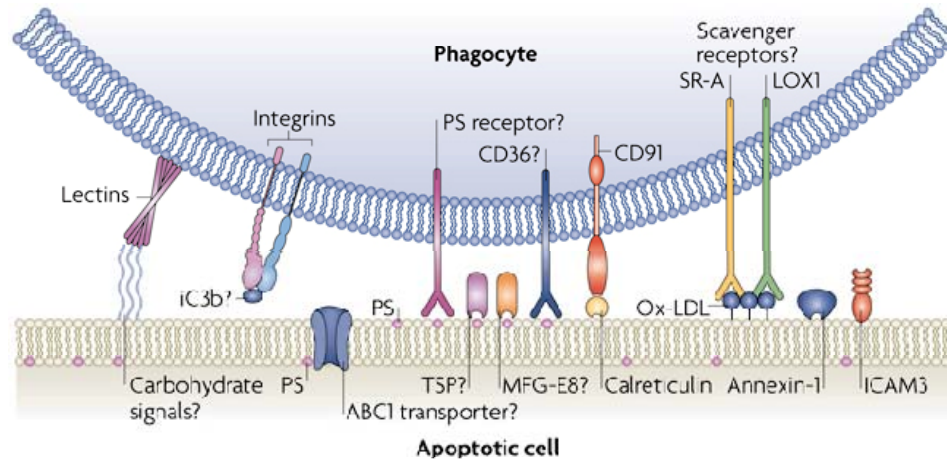
COPI coated vesicles
(Cellular component)

Intra-molecular oxido reductase
and Peptido-reductase activity
(Molecular function)



GO annotation results

Late lactation milk (day 250)



Nature Reviews | Molecular Cell Biology

DNA dependent DNA replication
Regulation of signal transduction
Regulation of apoptosis
(Biological process)

Peptide receptor activity/G-protein coupled
(Molecular function)

Proton transporting ATP synthase complex
Coated vesicle
(Cellular component)



Ubiquitously expressed genes

Transition milk

Late lactation milk

~8,000 genes

Intra-cellular organelles/intrinsic membrane of cells

- Genes expressed in a specific stage of the lactation were localized in extracellular matrix or vesicles



Statistical analysis

4359 genes significant change in expression
($p \leq 0.05$ and FDR $q \leq 0.2$)

Transition milk (day 15)



High expression
501 genes



Significant biological process GO terms

- circulation
- regulation of blood vessel size
- cell-cell adhesion
- homophilic cell adhesion



Statistical analysis

4359 genes significant change in expression
($p \leq 0.05$ and FDR $q \leq 0.2$)

Late lactation milk (day 250)

High expression
3858 genes

Significant biological process GO terms

- light stimulus
- visual perception
- calcium ion transport
- sensory perception
- neurophysiological process
- metal ion transport
- di-, tri-valent inorganic cation transport



Conclusions

- First to describe the comprehensive milk transcriptome in Holstein cows
- 48-51% of annotated genes are expressed in the bovine milk
- Most of the genes in milk were ubiquitously expressed in the two stages of lactation
- More genes contributed to the total mRNA population in the late lactation



Conclusions

- Milk cells have the ability to adapt different molecular functions according to the biological need of the animal
- This study provide a insight into the biology of lactation in cow and many future research avenues on the bovine lactome.



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THANK YOU !

