# A potential role for microRNAs in the regulation of onset of milk secretion

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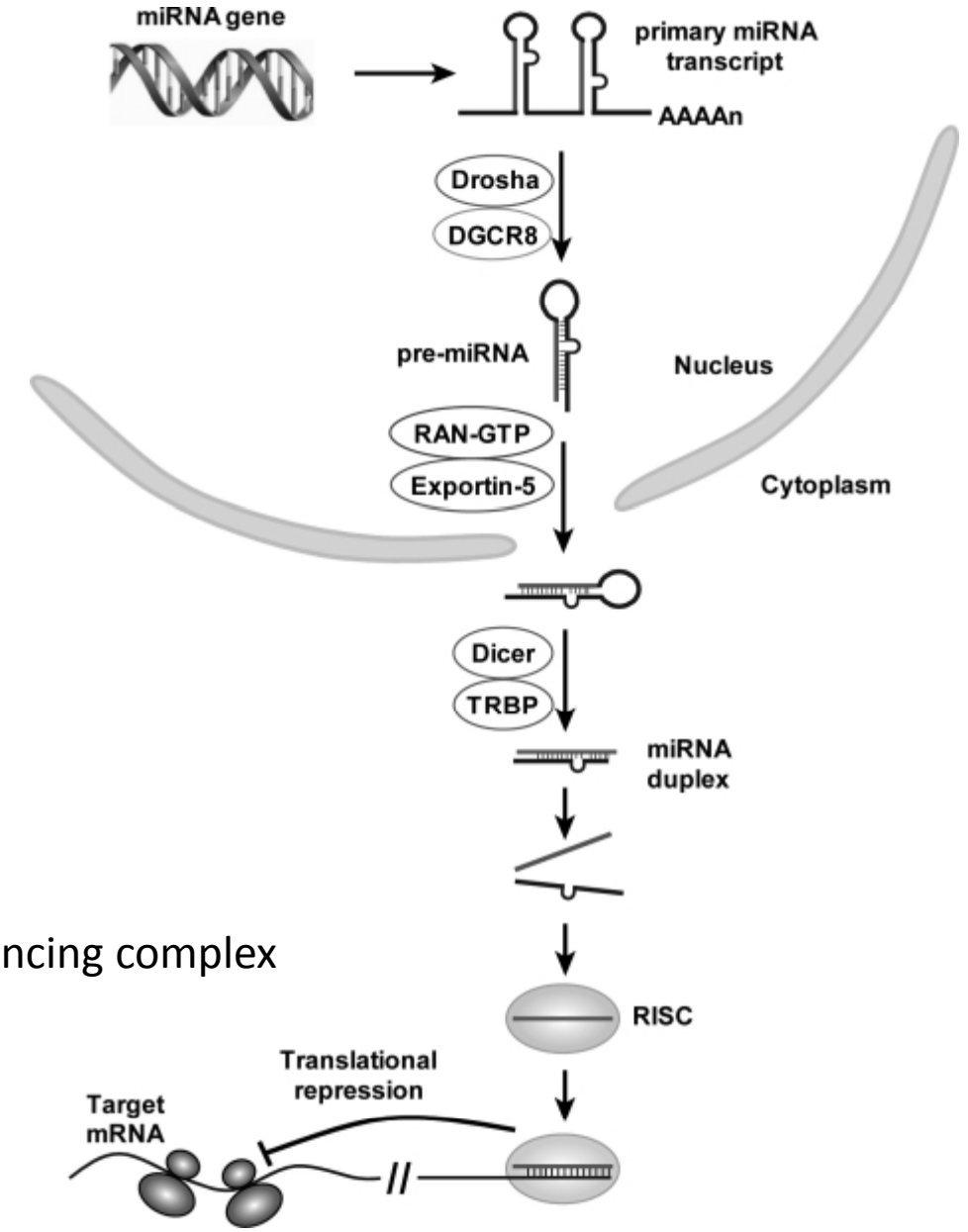
# Organization of talk

- Definition of microRNAs
- How to approach miRNA's when you don't know anything
  - mRNA/miRNA expression profiling
  - Validation of results
  - Target prediction
  - Functional studies

**Micro-RNA:** A tiny piece of small non-coding RNA, about 21 to 22 bases in length, that binds to matching pieces of messenger RNA to make it double-stranded and decrease the production of the corresponding protein

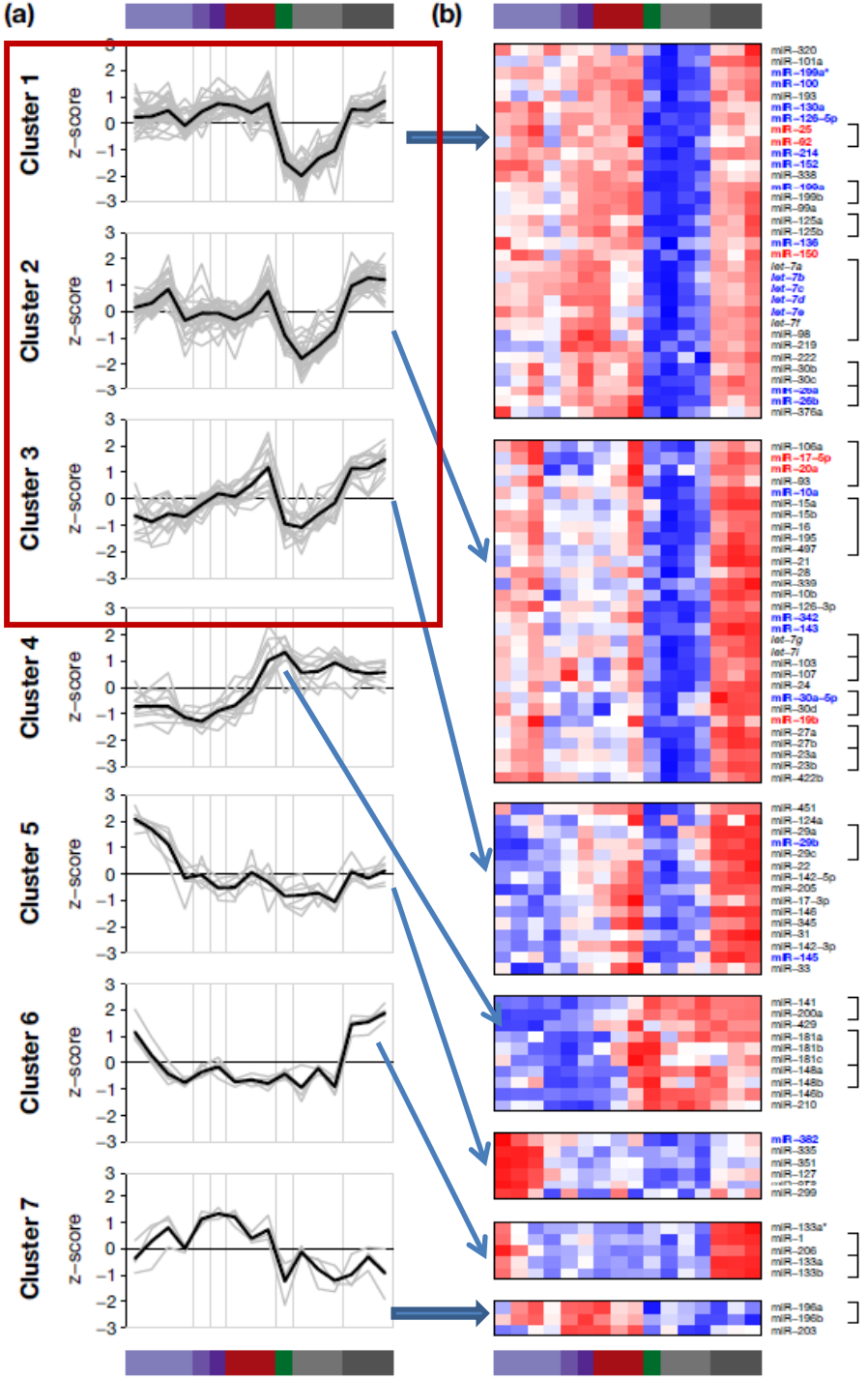



RISC – RNA induced silencing complex

**Can also have positive regulation, but we will focus on negative regulation only**

Exp Mol Med. 2010; 42(4): 233–244.

## *Caldas: BMC Genomics 2009, 10:548*




**Cl 1** Puberty, maturity & early gestation

Luminal breast cancer

Basal breast cancer

**Cl 2** Before puberty & late gestation

**Cl 3** Late gestation

**Cl 4** Lactation & early involution

**Cl 5** Early development

**Cl 6** Late involution

**Cl 7** Early development & gestation

**miRNA expression during mammopoiesis is highly correlated.** Seven clusters obtained by model-based clustering are displayed in separate panels. (a) Grey lines represent the standardized log2 expression profiles of individual miRNAs. Black lines correspond to mean cluster profiles. (b) Heatmaps of individual clustered miRNAs. Red and blue indicate high and low standardized log2 expression, respectively. Within each cluster panel, miRNAs are ordered according to miRNA families with identical seed sequence (position 2-8) (indicated by brackets). miRNAs associated with basal or luminal breast cancer are highlighted in red and blue, respectively. Individual panels correspond to the seven clusters in (a).

- Juvenile
- Puberty
- Mature virgin
- Gestation
- Lactation
- Early involution
- Late involution

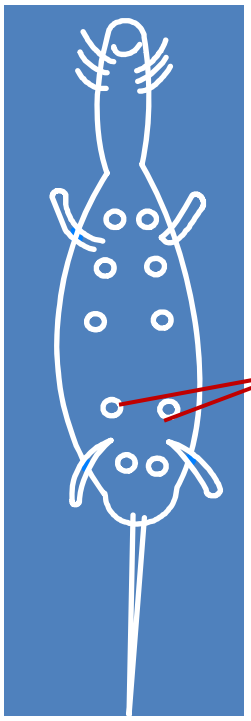
***Hypothesis:*** *microRNAs are critical regulators of normal mammary gland development and they may regulate some aspects of secretory activation such as glycolysis and lipid synthesis pathways*

**Aims**

- To assess the expression pattern of miRNA and mRNA in mouse mammary epithelial cells during secretory activation.

- Identification and functional validation of miRNAs regulating glycolysis and lipid synthesis pathways in the mammary gland.

# Expression profiling methods



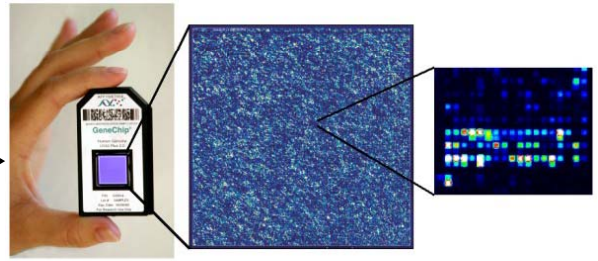
4 mice/ time-point
P14 & L2

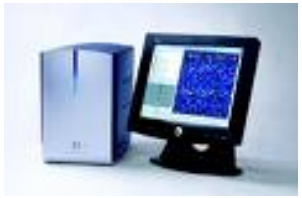
MEC digestion buffer –
Collagenase A &
Trypsin

**Adipose – depleted MECs**

**Trizol RNA isolation**


1. **Mouse Gene 1.0 ST Array** (28,853 genes)
2. **GeneChip miRNA array** (422 mouse miRNAs)





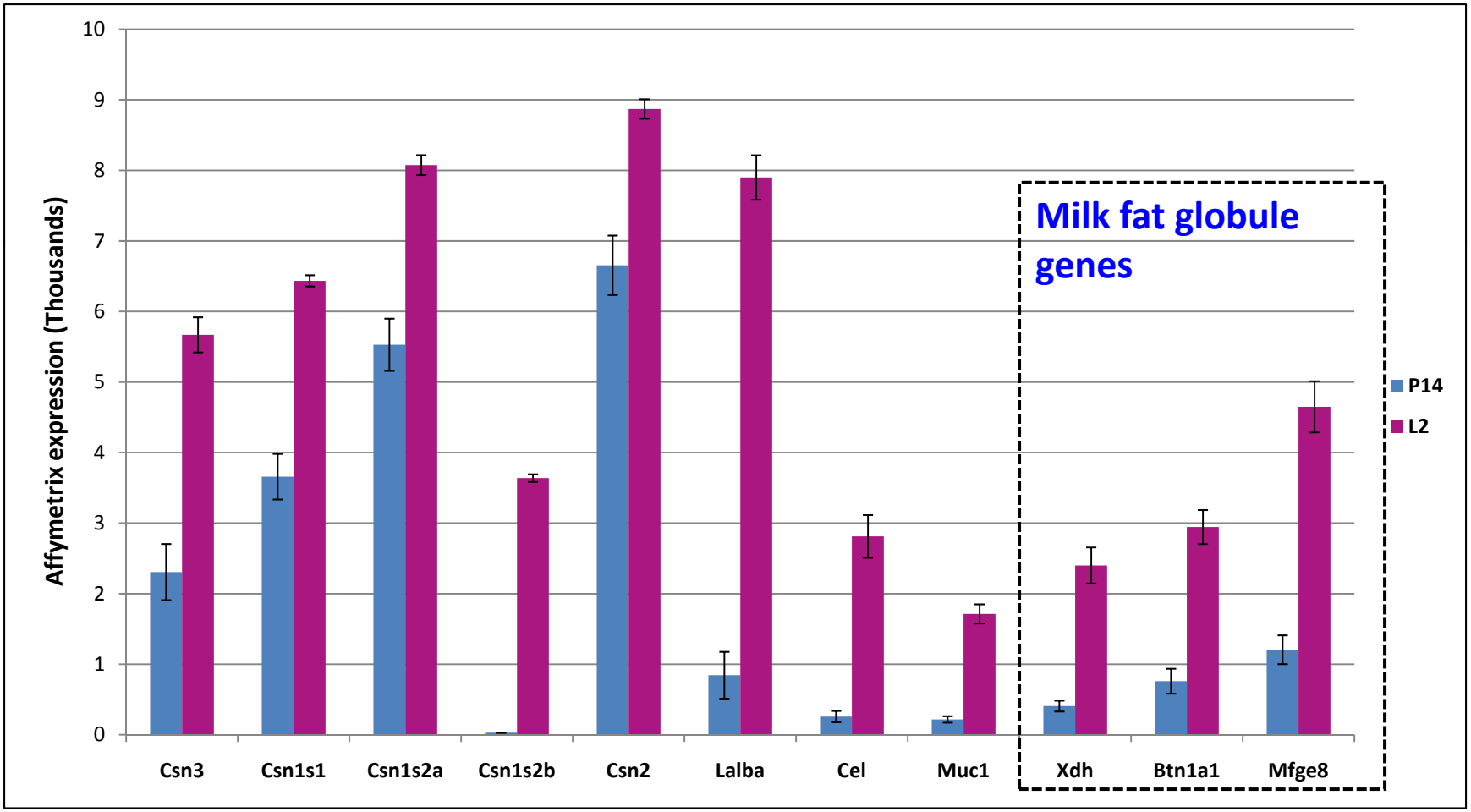

**Data Analysis & Interpretation**

# Differentially expressed mRNA's in MECs

| | P14 > L2 | P14 < L2 | Total |
|---|---|---|---|
| mRNA P<0.05 (FC>1.3) | 2,902 (2,420) | 2,604 (1,391) | **5,499 (3,806)** |

Corrected p-values – adjusted by multiple testing using BH-False Discovery Rate

# Expression of milk protein genes



# Lipid synthesis gene expression – WTL vs MECs

| Transcripts Id | PS ID | GS | WTL | | MEC | |
|---|---|---|---|---|---|---|
| | | | FC (L2/P14) | p-value | FC (L2/P14) | p-value |
| **Glucose Transport** | | | | | | |
| 10507594 | 93738_at | **Slc2a1** | 2.81 | **0.0009** | 3.27 | **0.0043** |
| 10481759 | 101397_at | **Slc2a8** | 1.33 | **0.0007** | 1.37 | **0.0125** |
| **Pentose phosphate shunt** | | | | | | |
| 10605338 | 94966_at | **G6pdx** | 0.90 | 0.1970 | 3.04 | **0.0043** |
| 10521984 | 101294_g_at | **G6pd2** | 1.70 | **0.0032** | 2.00 | **0.0106** |
| 10413542 | 101964_at | **Tkt** | 0.95 | 0.5935 | 2.20 | **0.0163** |
| **Fatty acid and triglyceride synthesis** | | | | | | |
| 10379153 | 160546_at | **Aldoc** | 2.65 | **0.0005** | 5.67 | **0.0211** |
| 10547830 | 99566_at | **Tpi1** | 0.67 | **0.0105** | 1.51 | **0.0273** |
| 10460400 | 93308_s_at | **Pcx** | 1.32 | **0.0310** | 5.36 | **0.0156** |
| 10438262 | 162358_i_at | **Slc25a1** | 2.32 | **0.0009** | 10.87 | **0.0018** |
| 10391146 | 160207_at | **Acly** | 1.65 | **0.0016** | 4.85 | **0.0063** |
| 10393970 | 98575_at | **Fasn** | 1.20 | **0.0272** | 6.59 | **0.0080** |
| 10507539 | 95642_at | **Elovl1** | 3.05 | **0.0006** | 3.72 | **0.0043** |
| 10444420 | 93720_at | **Agpat1** | 4.21 | **0.0003** | 2.05 | **0.0082** |
| 10386473 | 93264_at | **Srebf1** | 1.25 | 0.2044 | 1.73 | 0.0825 |
| 10565609 | 160306_at | **Thrsp** | 0.76 | **0.0181** | 4.02 | **0.0259** |
| 10572130 | 95611_at | **Lpl** | 0.99 | 0.9549 | 7.76 | **0.0110** |
| 10467979 | 94058_r_at | **Scd1** | 1.13 | 0.1741 | 5.90 | **0.0038** |
| 10467979 | 94056_at | **Scd1** | 1.20 | **0.0102** | 5.90 | **0.0038** |
| 10467979 | 94057_g_at | **Scd1** | 1.10 | 0.0744 | 5.90 | **0.0038** |
| 10463355 | 95758_at | **Scd2** | 1.45 | **0.0365** | 7.22 | **0.0043** |
| 10463355 | 162077_f_at | **Scd2** | 1.37 | **0.0343** | 7.22 | **0.0043** |

All lipid synthesis genes are significantly up regulated in Lactation day 2 relative to pregnancy day 14 in MECs and also show a higher fold change L2/P14 relative to WTL from mammary gland. Further, in WTL a few genes – Spot14, Tpi show higher expression in P14 relative to L2.

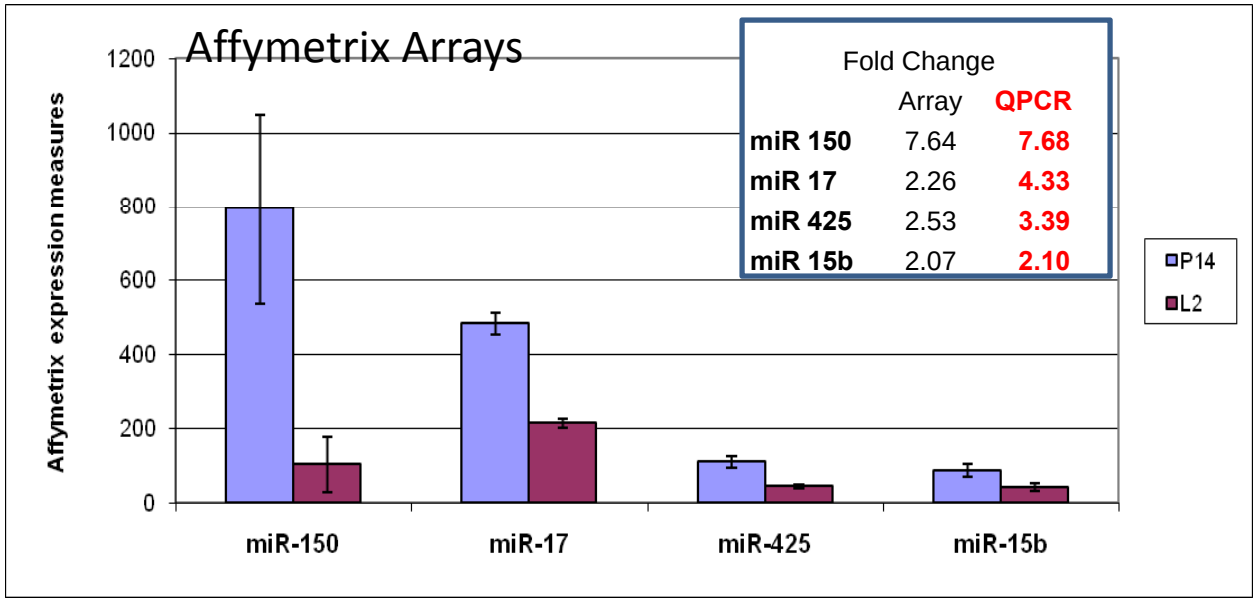
WTL – Whole tissue lysates
MEC – Mammary epithelial cells

# Differentially expressed miRNAs

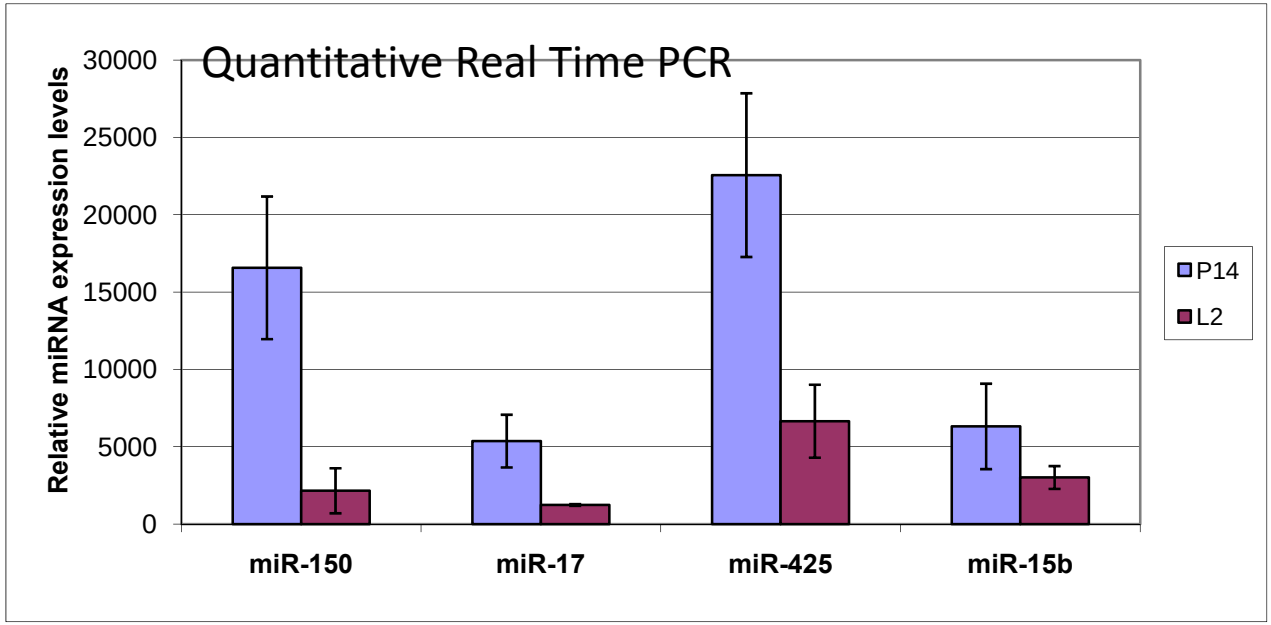
| | **P14 > L2** | **P14 < L2** | **Total** |
|---|---|---|---|
| miRNA P<0.2 (FC>2) | 34 (20) | 18 (7) | **52 (27)** |

Corrected p-values – adjusted by multiple testing using BH-False Discovery Rate

# miRNA RT-PCR validation




| Fold Change | Array | QPCR |
|---|---|---|
| miR 150 | 7.64 | 7.68 |
| miR 17 | 2.26 | 4.33 |
| miR 425 | 2.53 | 3.39 |
| miR 15b | 2.07 | 2.10 |



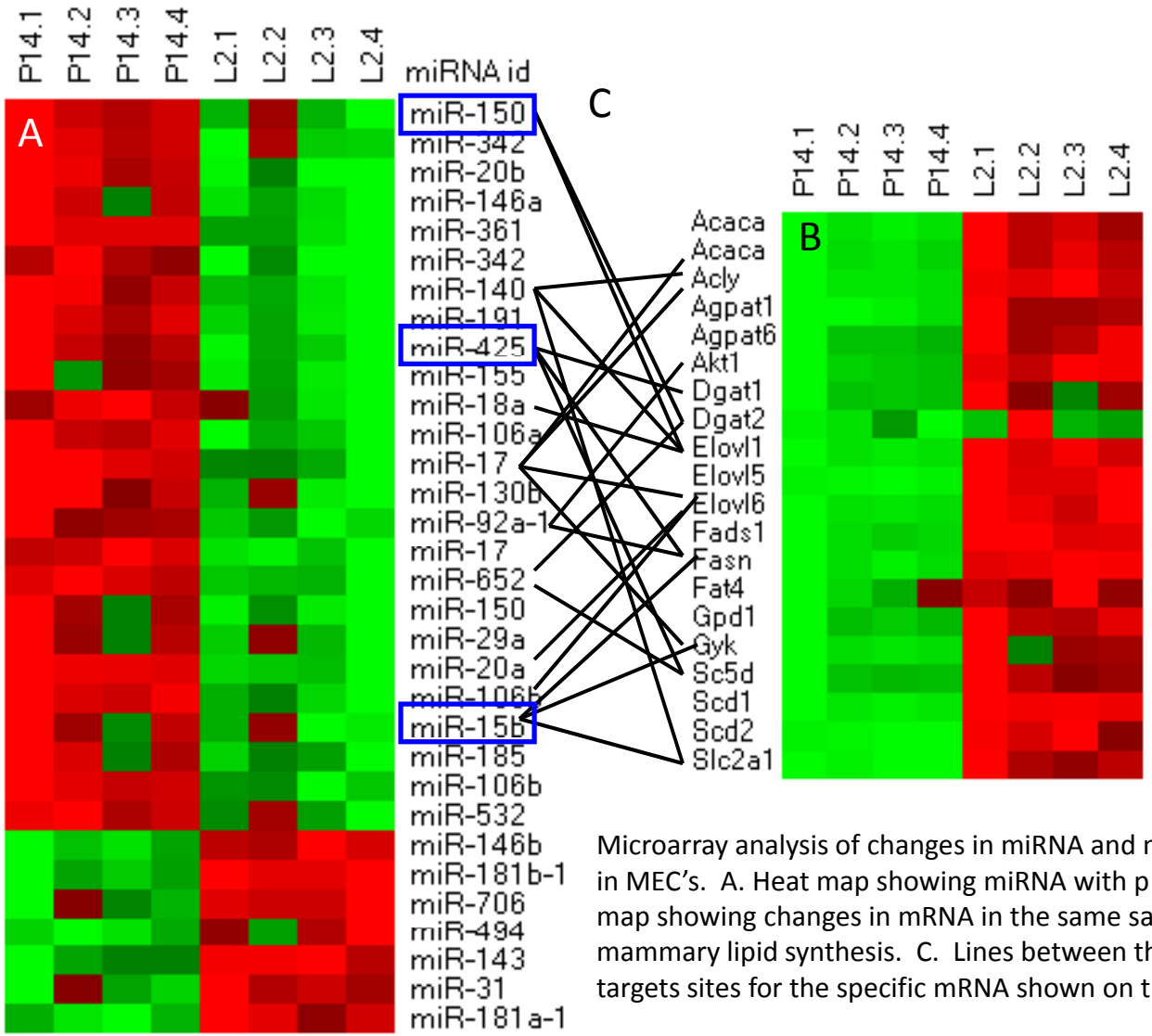

Comparison of results from P14 and L2 from the microarray QPCR data for selected miRNA's.
All these miRNA's **decrease more than 2 fold between late pregnancy and lactation**.

# Predicted miRNA targets and differentially expressed mRNAs

| DE mRNA targets → | DE mRNAs (FC>1.3) | | | |
|---|---|---|---|---|
| | miRANDA targets | | TargetScan | |
| DE miRNA direction ↓ | P14 > L2 | P14 < L2 | P14 > L2 | P14 < L2 |
| P14 > L2 (correlation) | 1,173 (48.5%) | **610 (43.9%)** | 406 (16.8%) | **208 (15%)** |
| P14 < L2 (correlation) | **604 (25%)** | 288 (20.7%) | **246 (10.2%)** | 78 (5.6%) |

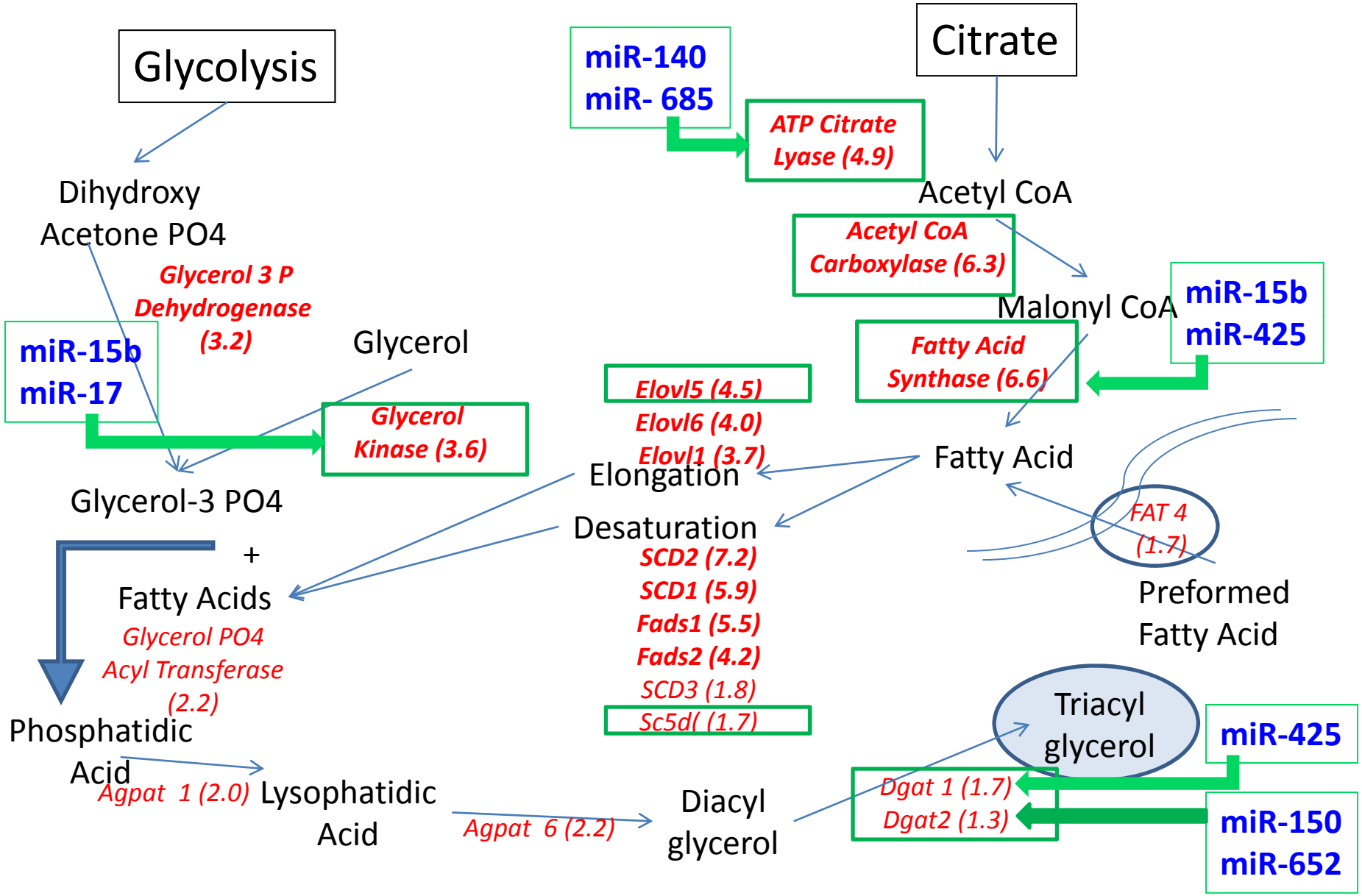
Correlation - % of differentially expressed genes

# Heatmap of miRNAs regulating lipid synthesis genes




Microarray analysis of changes in miRNA and mRNA expression between P14 and L2 in MEC's. A. Heat map showing miRNA with $p < 0.2$ and fold change > 2.0. B. Heat map showing changes in mRNA in the same sample for selected genes involved in mammary lipid synthesis. C. Lines between the two heat maps indicate genes with targets sites for the specific mRNA shown on the left.

# Fatty Acid and Triglyceride Synthesis



# Conclusions

- All milk protein and lipid synthesis genes known to be involved in secretory activation were significantly upregulated during lactation in the MECs much more than in WTL.
- Approximately 39% (164) of the mouse miRNAs present in the GeneChip were expressed in at least one of the two stages of mammary gland development of which 52 miRNAs were differentially expressed.
- miRNAs potentially regulating critical genes in the lipid synthesis pathway have been identified.

- *In vitro* functional assays for miR-15b which is predicted to target Fasn, Gyk, Glut1 and Adph besides others is currently underway in primary mammary epithelial cells.

# Acknowledgements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