

# Milking frequency modifies DNA methylation at a CSN1 regulatory region in the bovine mammary gland

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*EpigRAni project funded 2010-2013*

# Background

- Milk production is not only genetically defined
- Long term effects :
  - Nutrition, animal health, livestock conditions...
  - Milking frequency affects milk yield: once daily milking (1X) reduces milk production
- Reductions in milk production during mammary involution<sup>1</sup> or in the case of mastitis<sup>2</sup> have been related to a decrease in *CSN1S1* gene expression and associated with an increase in DNA methylation at one of the distal regulatory regions of the gene, located close to two Prolactin Responsive Elements (Stat5RE)

## Aims of the project

- What are the consequences of once daily milking in the cow, on *CSN1S1* gene expression and on DNA methylation profiles around this gene ?
- Which mechanisms can explain the long term effects induced by unilateral once daily milking (1X) versus twice daily milking (2X)?



<sup>1</sup> Singh et al (2010) *J. Mammary Gland Neoplasia*

<sup>2</sup> Vanselow et al (2006) *J. Mol. Endocrinol.*

# Animals & Experimental Design

**Eight Holstein heifers**  
Well balanced mammary development  
between left and right half udders



Calving

53 days in milk (2X)

P1, 2X 2X

P2, 1X 2X

P3, 2X 2X

Daily Milk Production/half udder

Biopsies

RNA

IHC1

DNA

IHC2

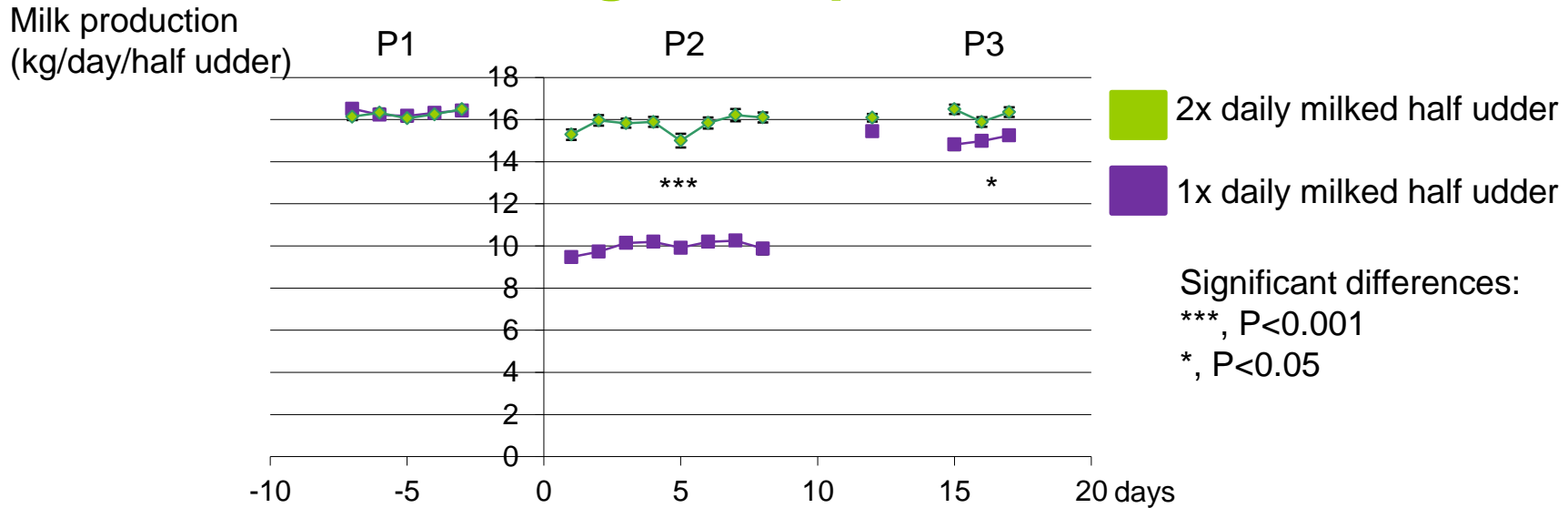
MEC

IHC3

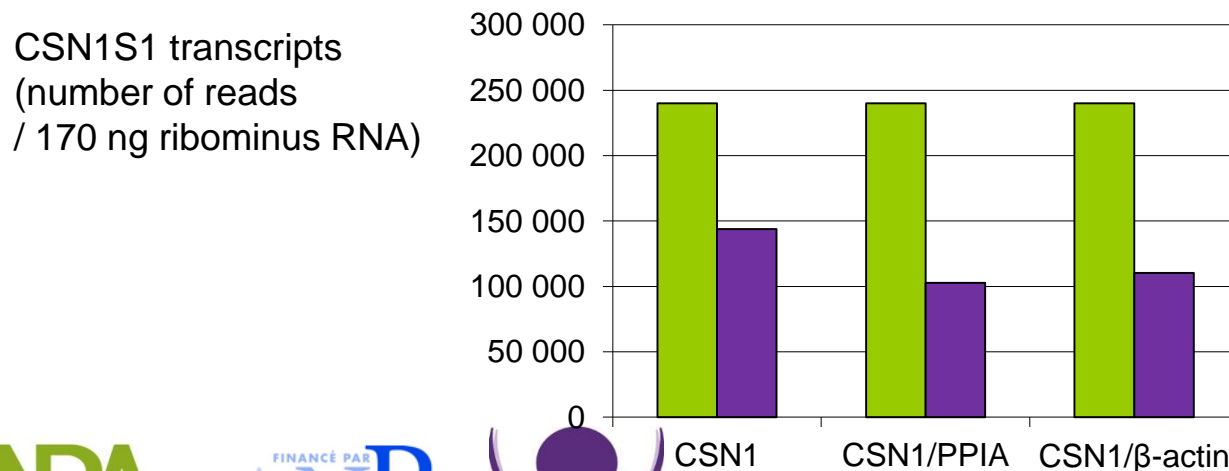
EM

protein

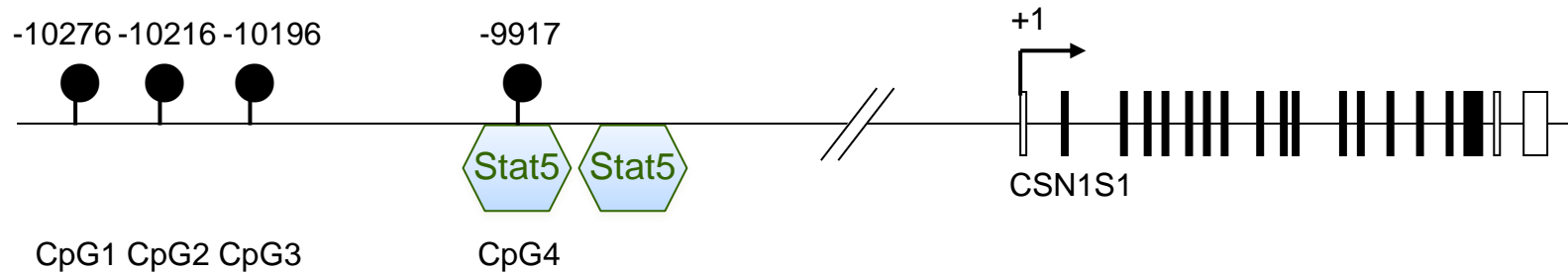
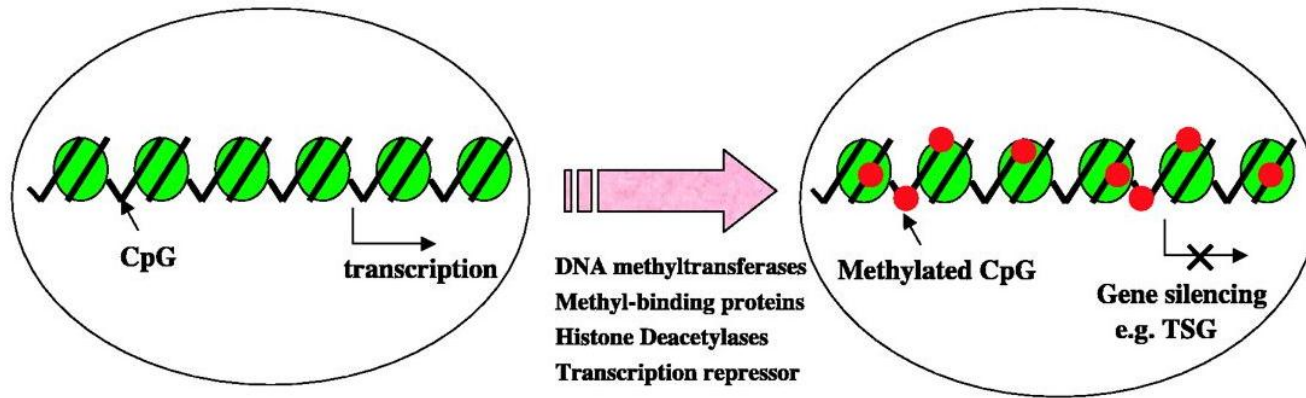
# Once daily milking reduces both milk production and *CSN1S1* gene expression



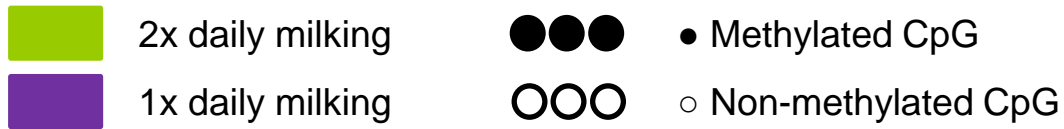
Marion Boutinaud, PEGASE



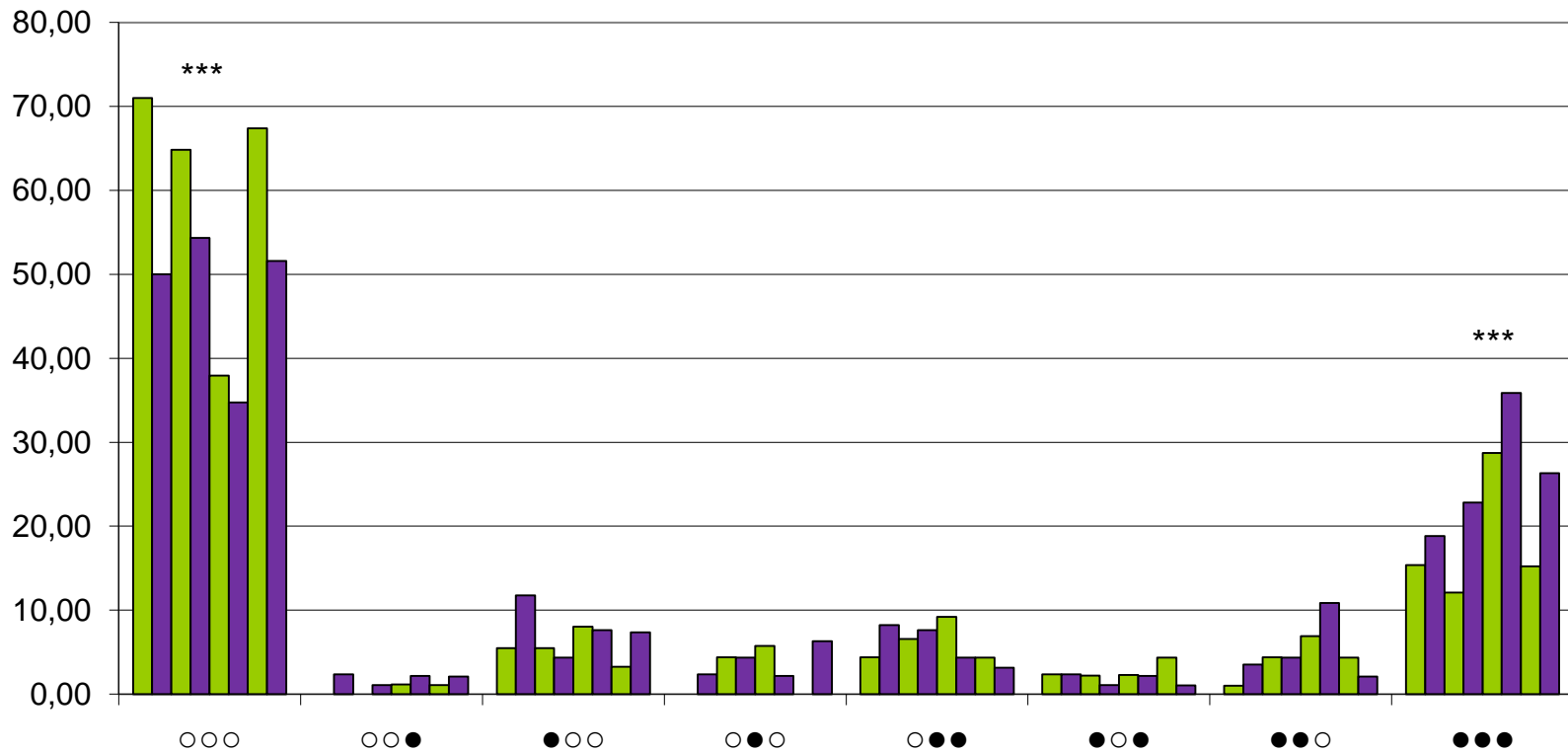
# DNA methylation at a distal regulatory region of *CSN1S1* gene



# DNA Methylation patterns « CpG1-CpG2-CpG3 » for different cows during lactation

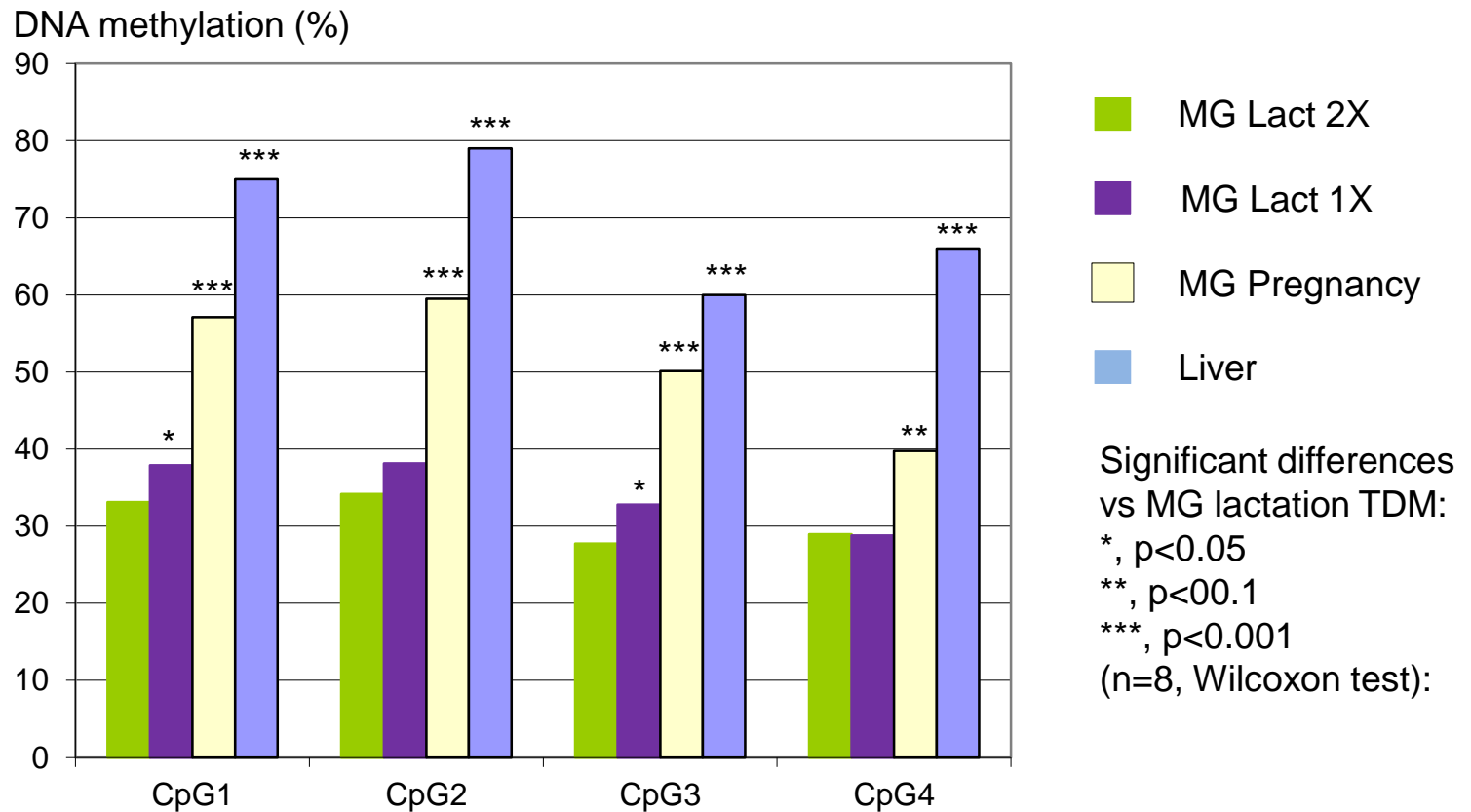


Number of clones for each profile (% , n=96)



\*\*\*, p<0.0001

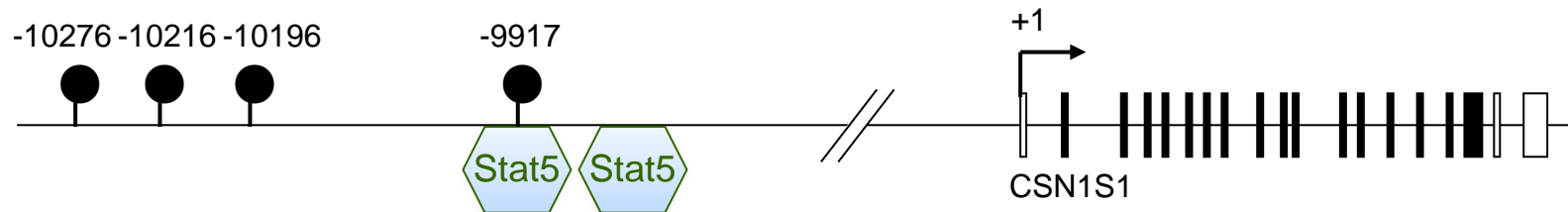
# 1X vs 2X induces a significant increase in DNA methylation at two CpG sites



# Conclusions

Unilateral once daily milking induces:

- A significant 37% decrease in milk production. A long term effect with a 7% decrease in milk production when 2X daily milking is resumed
- A significant 50 % decrease in CSN1S1 mRNA accumulation in the MG
- A significant 12 to 25 % increase in DNA methylation at two CpG sites in a distal region of CSN1S1. A methylation state expected to be maintained until DNA methylation marks are passively erased through cell division.
- No modification in the methylation of a CpG site located within a distal Stat5RE. From preliminary experiments, it is noteworthy that this Stat5RE has a lower affinity for Stat5 as compared to its neighbour and that its methylation state does not affect Stat5 binding *in vitro* (EMSA).



CpG1 CpG2 CpG3

CpG4



INRA

FINANÇÉ PAR  
ANR



APIS-GENE

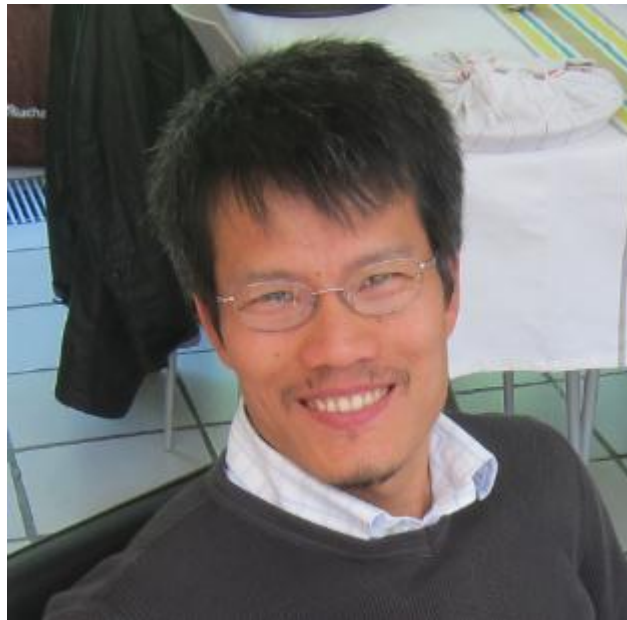


## Further questions

- What is the precise role played by this distal region and its epigenetic status in *CSN1S1* gene expression ?
- Epigenetic modifications in this region have been described<sup>1</sup> during involution, in relation with alterations in Stat5 signaling which occur 24-36h post milking
- Through epigenetic modifications, is the chromatin structure around Stat5RE modified ? and in turn can it alter the cooperation with other Stat5RE and Stat5 tetramer association ?

# Thank you for your attention !

A talented and really nice postdoc looking for a position!



**Minh Nguyen (PhD)**

Molecular biology

Animal genetics

