Eloise Lynch – SRF Vacation Scholarship Report 2015

Cyclical DNA methyltransferase expression across multiple timescales in the uterus of the Siberian hamster (*Phodopus sungorus*)

Introduction

It is becoming clear that epigenetic modifications, such as DNA methylation exhibit dynamic and reversible changes. Our understanding of a role for epigenetic modifications, such as DNA methylation, for timing biological rhythms is in its infancy. It has recently been found that DNA methylation in the hypothalamus plays a role in regulating the internal representation of seasonal time (Stevenson and Prendergast, 2013). A number of studies have illustrated increased DNA methylation in the progesterone receptor (PR) promoter in the brain is regulated by estradiol (Schwarz et al., 2010) and has detrimental consequences for quality of maternal care and receptivity to male advances (Matsuda, 2014). I am interested in whether similar epigenetic modifications occur in female reproductive tissues, particularly the uterus. I tested the hypothesis that epigenetic modifications are also responsible for controlling reproductive rhythms across a number of timescales in peripheral reproductive tissues. Using a seasonally breeding animal model, the Siberian hamster (*Phodopus sungorus*), I examined the naturally occurring seasonal and estrous variation in mRNA expression of DNA methyltransferase (*dnmt*) in the uterus

Methods

All animals were housed in polypropylene cages, provided cotton nesting material and food and water *ad libitum.* All procedures were approved by Institutional Animal Welfare and Ethics Review Board.

Seasonal changes in dnmt *expression*

Adult female Siberian hamsters (n=8, 3-8 months old) were maintained in long day (LD) conditions. Another group (n=10) were transferred into short day (SD) conditions. After 8 weeks all individuals were sacrificed by cervical dislocation. The uteri were removed, frozen in powdered dry ice and stored at -80^{o}C until further use.

Changes in dnmt *expression across the estrous cycle*

Adult female Siberian hamsters (n=33) were maintained in LD conditions. After 8 weeks all individuals were sacrificed by cervical dislocation. Stage of estrous cycle was determined by uterine mass and plasma prolactin concentration. The uteri were removed, frozen in powdered dry ice and stored at -80^{o}C until further use.

Changes in dnmt *expression within 24 hours*

Adult female Siberian hamsters (n=21) were ovariectomised and maintained in LD conditions for 8 weeks to ensure reduction in the levels of naturally occurring ovarian steroid hormones. Animals received a 100µl estrogen and progesterone bolus (E2P4) through an intraperitoneal injection at 1700. Control hamsters received 100µl of sterile vegetable oil (OIL). The uteri were removed, frozen in powdered dry ice and stored at -80oC until further use.

Quantification of dnmt3a *and* dnm3b *mRNA expression*

RNA was extracted from tissue samples using Trizol as described by the manufacturer's protocol and stored at -20^{o}C until further use. First strand cDNA synthesis was carried out using Superscript III First Strand Synthesis Kit from Invitrogen, following the manufacturer's

instructions. RT-qPCR analysis was run in triplicate (samples from the estrous cycle were run in duplicate). PCR Miner (Zhao and Fernald, 2005) was used to calculate reaction efficiencies (E) and cycle thresholds (CTs). The expression of each target gene was compared to the expression of a reference gene (*gapdh*) and calculated using the formula 2^-(delta-deltaCt).

Results

Seasonal changes in dnmt *expression*

A t-test revealed that entry into SD conditions resulted in a significant increase in expression of *dnmt3a* (t=3.103, p<0.01; Fig1A) and *dnmt3b* (t=3.782, p<0.005; Fig1B).

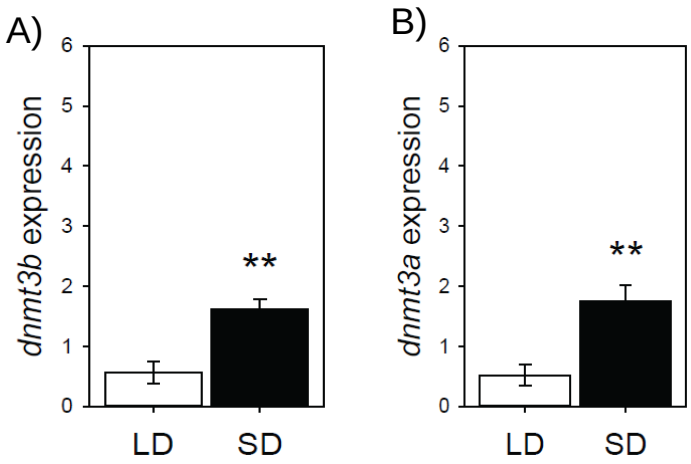

Figure 1 Mean expression (+SD) of A) *dnmt3a* and B) *dnmt3b* in the uterus of adult Siberian hamsters (n=18) in long day and short day conditions, relative to control genes *gapdh* and *βactin*. ** p < 0.01

Changes in dnmt *expression across the estrous cycle*

A one-way ANOVA revealed a significant difference in *dnmt3a* expression (F=3.362, p<0.05; Fig2A) across the estrous cycle, and LSD analyses showed that this significant decrease in *dnmt3a* expression occurred on transition from proestrus to oestrus (p=0.01). There was no significant variation in *dnmt3b* expression across the estrous cycle (F=1.644, p=0.21;Fig2B)

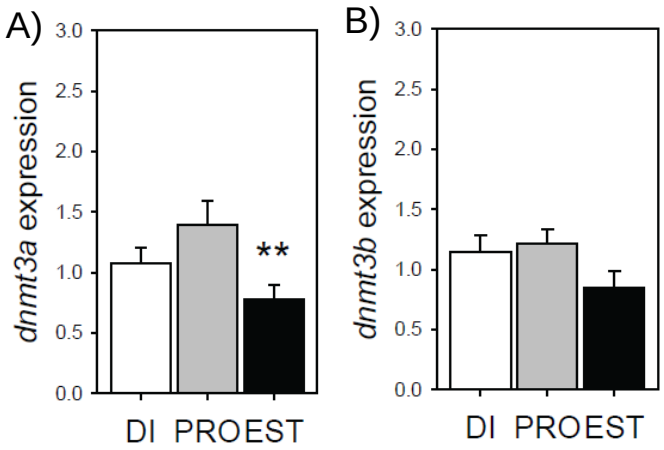

Figure 2 Mean expression (+SD) of A) *dnmt3a* and B) *dnmt3b* across the estrous cycle in the uterus of adult Siberian hamsters (n=33), relative to control genes *gapdh* and *βactin*. ** p < 0.01

Changes in dnmt *expression within 24 hours*

A one-way ANOVA revealed a significant difference in *dnmt3a* expression after administration of E_2P_4 ($F=8.424$; $P<0.005$; Fig3A). Dunnett's Method indicated that E_2P_4 induced a rapid inhibition in *dnmt3a* expression with a significant reduction after 12hr ($P<0.01$) and 24hr ($P<0.005$) compared to OIL treated females. Similarly, there was a significant difference in *dnmt3b* across treatment groups ($F=19.07$; $P<0.001$; Fig3B). E_2P_4 significantly reduced *dnmt3b* expression in uterine tissue 12hr ($P<0.001$) and 24hrs ($P<0.001$) after administration.

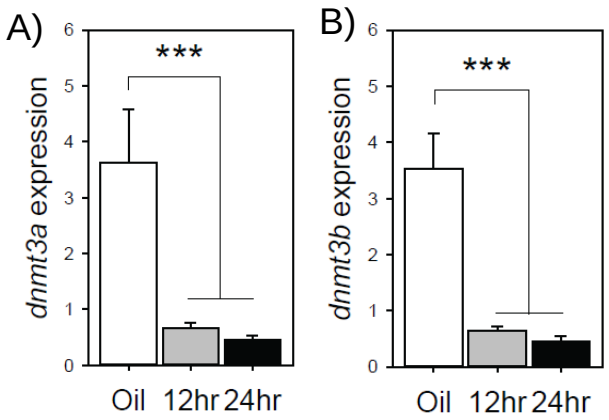

Figure 3 Mean expression (+SD) of A) *dnmt3a* and B) *dnmt3b* 12 and 24 hours post-injection with an E_2P_4 bolus in the uterus of adult Siberian hamsters (n=21), relative to control genes *gapdh* and *βactin*. *** $p < 0.001$

Discussion

I present novel and robust findings that *dnmt3a* expression is dynamic across a number of different timescales and propose that variation in DNMT3a is involved in the local timing of reproductive physiology in key tissues. These data have significant implications for our understanding of the potential effects of DNA methylation for fertility in a rodent species with direct applications for human reproductive health. Uncovering the mechanism that underlies this natural pattern could have a significant impact for developing effective long-term male contraceptives. I propose that epigenetic modifications are involved in molecular timing across multiple timescales and may represent an evolutionarily ancient clock mechanism.

References

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