Circadian gene expression in mouse uterus and liver

A.A. Lim[1], M.H. Johnson[2] and M.L. Day[1], [1]Department of Physiology and Institute for Biomedical Research, University of Sydney, N.S.W. 2006, Australia and [2]Department of Anatomy, University of Cambridge, CB2 3DY, United Kingdom.

The circadian rhythm of the suprachiasmatic nucleus (SCN) of the brain is entrained by light. Upon input of light, the circadian rhythm is generated as follows: the transcription factors CLOCK and BMAL1 heterodimerise to upregulate transcription of the genes *Per1-3* and *Cry1-2*. PER and CRY proteins heterodimerise, then translocate to the nucleus where they down-regulate CLOCK/BMAL1 activity. This reduces *Per* and *Cry* transcription and PER and CRY decay, releasing the inhibition on BMAL1. Eventually BMAL1 levels increase, initiating the next 24 h cycle of transcription. The circadian clock in the SCN coordinates circadian clocks in peripheral tissues (Reppert & Weaver, 2002). In the female reproductive tract and embryo, timers control when developmental events occur (Johnson & Day, 2000). It is possible that the circadian clock acts as one such timer as we have shown that the circadian genes, *Per1-3, Cry1-2, Bmal1* and *Clock*, are expressed in the female reproductive tract (uteri and oviducts) and in preimplantation embryos of the mouse (Johnson *et al.*, 2002). To determine whether a circadian clock exists in the uterus, we have quantified mRNA using real-time PCR.

Female MF1 mice were housed on a 12/12 h light/dark cycle. Liver and uterine tissues were obtained from mice euthanised by cervical dislocation at circadian times 0, 4, 8, 12, 16 and 20 of the proestrous, oestrous and dioestrous periods of the oestrous cycle. Whole tissues were snap frozen in liquid nitrogen and total RNA was purified. The RNA concentration was determined by RiboGreen assay, then reverse transcribed to cDNA. Expression of the circadian genes *Per2, Cry1, Bmal1* in the cDNA was then quantified by real-time PCR using SYBR Green I. Standard curves were run for each gene (β-*actin, Per2, Cry1, Bmal1*) using cDNA. From each standard curve the amount of each gene expressed was calculated and then normalised to β-*actin*.

Timed liver cDNA samples showed changes in circadian gene expression similar to that in the literature (Lee *et al.*, 2001). This confirmed the use of the real time PCR protocol for use on uterine samples. In uterine cDNA, preliminary results suggest that *Per2* and *Bmal1* expression change with a circadian rhythm. Further research will determine whether a circadian clock exists in other periods of the oestrous cycle.

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