One long term objective of this project is to understand the development of the ability of organs peripheral to the suprachiasmatic nucleus (SCN) to synchronize with the external environment. The first aim supports this objective with a systematic analysis of when during development an entrainable circadian rhythm is present in the developing eye, ear, nose, heart, and pronephros of the model organism, *Xenopus laevis*. We first test for the onset of circadian rhythm in each organ within the context of the embryo by assaying for rhythmic expression of circadian genes in a light/dark (LD) cycle. Next, the ability of each organ to intrinsically generate circadian rhythms is tested by assaying for rhythmic circadian gene expression in organs cultured in vitro in constant darkness (DD). Lastly, the ability of each organ to directly entrain to light is tested by culturing organs in vitro for two days in LD followed by three days in a DL cycle and assaying for rhythmic gene expression that correlates with the anti-phase light regime. Quantitative Real Time PCR will be used to measure gene expression in all these experiments.

**Intellectual merit of the proposed activities:** Little is known about how rhythmic gene expressions among organs in the embryo or adult are synchronized. The experiments outlined above will provide the foundation for experiments involving organ co-culture and transplantation to address how synchronization between organs develops in the embryo.

Another long term objective is to ask whether circadian rhythm is necessary for proper organ development or whether circadian genes are used to regulate ultradian rhythms that regulate developmentally timed events in specific organs. One well characterized ultradian rhythm occurs during somite segmentation in the embryo, but although three gene families involved in the timing of segmentation have been characterized, the pacemaker of somite segmentation has not. The second aim supports our long term objectives by following up on preliminary results that show that depletion of xNOCTURNIN (controlled by the circadian oscillator) can decrease the number of somites in the early embryo. We propose to either deplete or overexpress xNOCTURNIN protein in one cell of two celled embryos and analyze for somite formation by immunohistochemistry to test whether xNocturnin plays a role in somite segmentation.

**Intellectual merit of the proposed activities:** Proper somite segmentation requires the maintenance of a two hour ultradian rhythm. The pacemaker for this process has yet to be characterized and our investigations will address whether a circadian oscillator controlled gene (xNocturnin) plays a role in this process.

**Broader Impact:** This proposal is a vehicle for providing an opportunity for undergraduates to become immersed in basic research full time for ten weeks during the summer (impact on 8-12 undergraduate students over three years). The University of Wisconsin at Whitewater has a strong commitment to undergraduate research. Also, programs are in place to support undergraduate research through small intramural grants awarded to students as well as programs that support hands on lab research experience for minority students (WISCAMP and McNair programs). This proposal builds on these programs and provides support for students that have been trained in the laboratory in the fall and spring semesters to continue their research full time for 10 weeks of the summer and become immersed in their project. Students will be exposed to basic concepts of developmental and circadian biology while learning both embryological and molecular techniques including microdissection and tissue culture, microinjection, molecular genetics (protein depletion using morpholinos and overexpression of protein by mRNA injection), cloning and building specific molecular constructs, and quantitative Real Time PCR.