I. We hypothesize that organs peripheral to the SCN develop an entrainable circadian rhythm during early to late tadpole stages in *Xenopus laevis*.

Question A. When can rhythmic gene expression be detected in developing organs in the context of the whole organism (in vivo)?

Organs (eye, ear, nose, heart, and pronephros) in embryos cultured in a LD cycle will be assayed at specific stages of development at six different times of day. We will assay for rhythmic expression of circadian clock associated genes using quantitative Real Time RT-PCR (qRT-PCR) to determine a developmental age when rhythmic gene expression is first present in each organ.

Question B. When during development are peripheral organs able to intrinsically generate circadian rhythms?

Rhythmic gene expression of an isolated organ in constant conditions is a hallmark of a circadian oscillator. Peripheral organs capable of rhythmic gene expression in vivo will be isolated and cultured in DD for two days. On the third day, we will analyze the temporal profiles of *xBmal1* and *xNocturnin* using qRT-PCR to determine if the organs can generate self sustaining circadian rhythms in vitro.

Question C. Are embryonic and adult organs in *Xenopus* light entrainable?

The peripheral organs of zebrafish are light entrainable, while the peripheral organs (other than the eye) of mice/rats/hamsters are not (Whitmore, 1998 and 2000; reviewed in Stratman and Schibler, 2006). Organs with established circadian rhythms will be isolated from both embryos and adults, cultured in LD for 2 days and then switched to DL for three days. Circadian clock gene expression will be analyzed as using qRT-PCR to determine if the organs were able to entrain to an alternative (antiphase) light cycle (DL).

**Background and Significance:** Be careful to only tell the reviewers the basic information about what you are studying, what is known and what is not known. Some background may be needed in this section or within the experimental design so reviewers can understand how your experiments will be performed, that support your predicted results, and that explain any pitfalls and how they can be overcome.

One way to approach writing this section of the grant is to first write a general outline of the material you think should be presented (rough draft). Then write up the preliminary data and experimental design sections. Afterward, return to the background and significance and write a more focused discussion of the relevant information necessary to understand your proposal.

**Preliminary Data:** A strong grant includes preliminary data that shows some proof of concept that your experiments are working. This does not mean that you will not be funded if you have no preliminary data, but that it will be harder. Be sure to highlight any student involvement in obtaining these preliminary results!

**Student Involvement and Impact Statement:** I included a statement on student involvement and impact in my preliminary data section. I outlined the types of techniques the students would learn, my experience in mentoring students, the involvement of students in some of the preliminary research and publications that included student authors, impact on student careers due to research experience that I had and related it to the broader impact. Other authors prefer to place the student involvement and impact as a separate section at the end including a rough timeline of the grant.
to highlight the importance of this aspect of the proposal as well as to emphasize why experiments might take a bit longer at a PUI. It probably depends somewhat on how the grant is written.

Link to example 1 (included within preliminary data)

Student Involvement and Impact

The experiments outlined above have already had a broad impact on fourteen undergraduate students and two local high school teachers in giving them the opportunity to learn how to do basic scientific research. We anticipate that with successful funding of this proposal we will have an even greater impact because it will allow undergraduate students to focus on their research projects during the summer without having to work a part time job and when they are less distracted by their studies (4 students/summer). Students will be exposed to basic embryological concepts such as cell and organ differentiation, differential gene expression, circadian and ultradian (developmental) timing, as well as cell-cell and cell-tissue interactions. They will gain experience in experimental design, microdissection and culture of embryonic organs, microinjection, qRT-PCR and immunohistochemistry. Students will have the opportunity to present their results at local, regional, and national meetings. In fact, the Curran lab won first prize at the UWW undergraduate research day three years in a row (Rachel Yanke (2006), Aaron Trow and Jessica Solis (2007), and Nicole Sarver (2008)). The same three students won the poster competition at the [j]regional meeting in years 2006-2008 as well. Sarah Meyer was a finalist this year (2009) and did the qRT-PCR analysis for Figures 2 and 3 above. The individual student projects will result in 1-2 publications. Students will have the opportunity to create figures and participate in writing and editing these publications.

On our campus we serve two minority groups. One is ethnic minorities and the other is disabled students. UWW has two programs that actively recruit and support minority students to work in research laboratories early in their undergraduate careers (WISCAMP and MacNair programs; Jessica Solis). Also, one of the objectives for UWW campus is to serve disabled students through facilities and services, thus the Curran lab is handicapped accessible. Jessica Solis and Annamarie Sieburns along with Joe Dodge and Brittany Bronson were involved in obtaining the unpublished portion of the in situ data outlined in Figure 1 (xCry1, xCry2, xClock, and xNAT). qRT-PCR results mentioned in Aim I for the ontogeny of circadian rhythm in the heart and ear were obtained by Lacmbouh Ade and Jessica Solis.