The Circadian Expression of Opsins in the Eye and Skin : A Comparison

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Introduction

Circadian Rhythms are the body's internal clock. At a cellular level, the clock operates through the oscillations in transcription and translation of 4 core transcription factors: Period (PER), Cryptochrome (CRY), BMAL1, and CLOCK[1].

Our circadian rhythm is synchronized to the light and dark cycles of the environment , thus, the detection of light in the environment is crucial. Light is primarily sensed by opsin proteins that convert a photic signal to an intracellular signal.

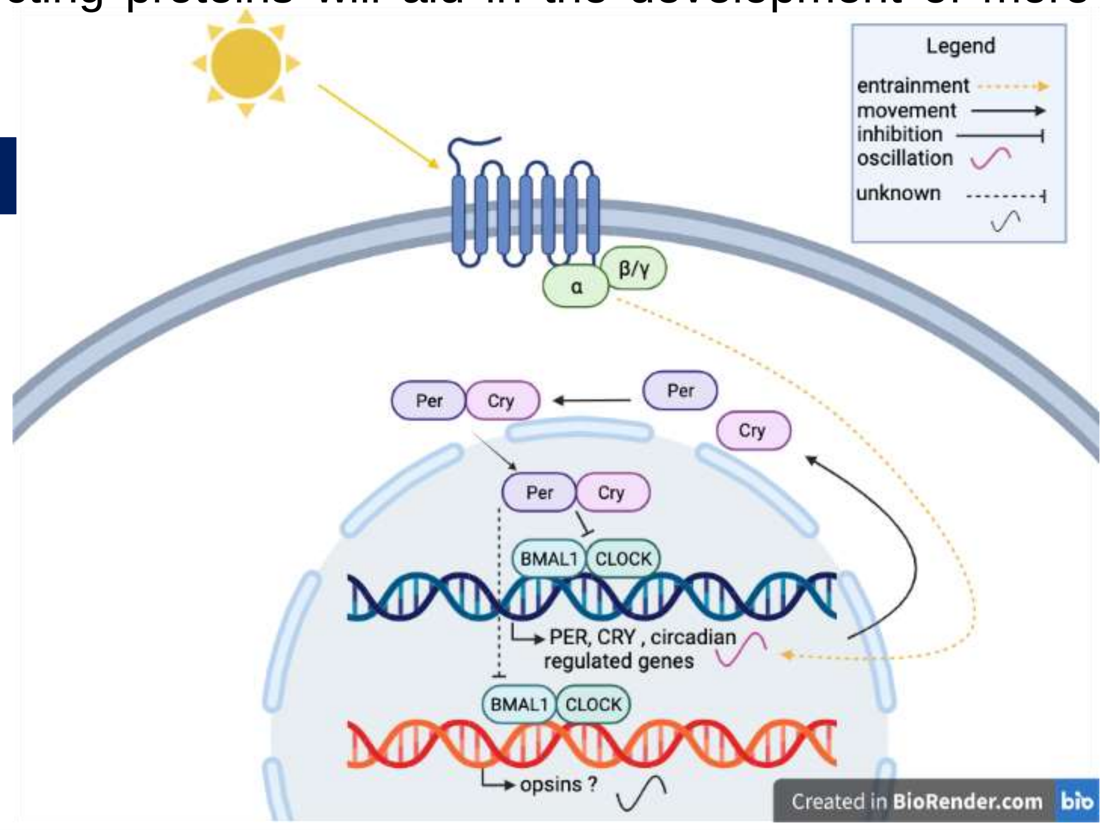
Melanopsin, expressed in intrinsically photosensitive retinal ganglion cells (ipRGC), is partly responsible for circadian photoentrainment.

Studies have demonstrated that opsins synchronize the circadian rhythms of an organism, however, if opsins show rhythmic expression is still being studied. Furthermore, opsins are expressed in the skin and brain where their function is poorly understood.

Some of the current treatments for circadian rhythm disorders are light exposure based. Better understanding of the way light is detected, and the regulation of light detecting proteins will aid in the development of more effective treatment.

OBJECTIVE

To determine if opsins are regulated in a circadian manner in the eye and skin.



Hypothesis

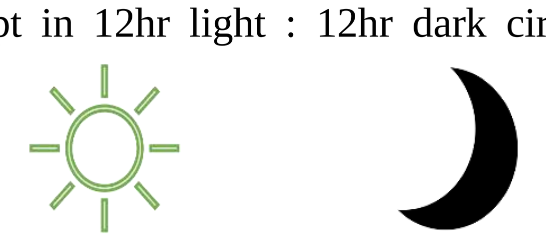
In peripheral tissues opsins have the same cyclic expression of mRNA over 24 hours as they do in the eye.

Materials and Methods

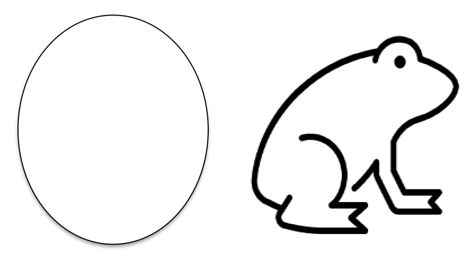
Xenopus Laevis embryos were reared in 12:12 hr. light: dark for 5 days and collected at pre-metamorphic stage 43/44 to extract RNA from eyes and tails and whole embryos and prepare cDNA.

Melanophore Cell Culture: Mex cells were cultured in 70% Leibovitz L-15 medium , 25% H_2O, 5% fetal bovine serum. Cells were kept in 12hr light : 12hr dark circadian rhythms and collected at the different times.

- GraphPad prism was used to create densitometry graphs complete statistical analysis



12 hours	12hours



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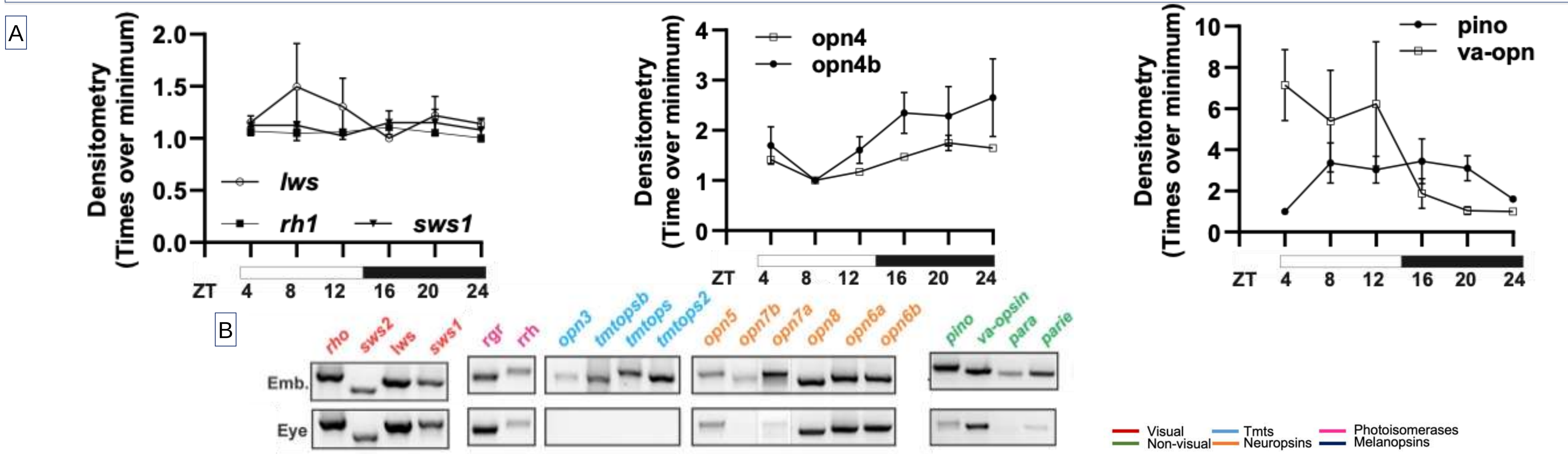
Results

Opsin Expression in the Eye



Opsin Expression in the Skin

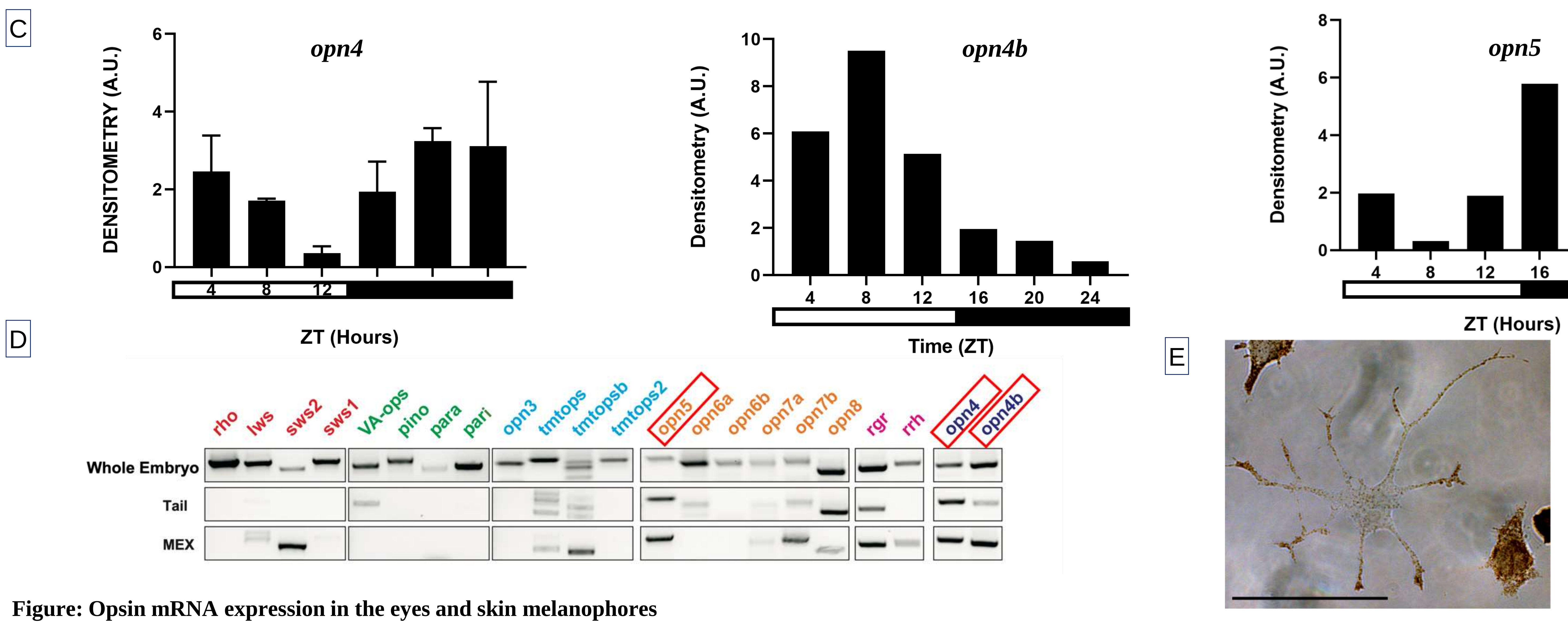


Figure: Opsin mRNA expression in the eyes and skin melanophores

A,C) RT-PCR densitometry in xenopus eye and melanophores represented by the mean ± sem. Eye densitometry is a repeat of 2 and biological N=1. Melanophore densitometry for opn4 is an N=2 and for opn4b and opn5 an N=1. Image J was used to quantify the densitometry of the RT-PCR bands.

B,D) Representative RT-PCR expression data of all the opsin genes found in *Xenopus laevis* genome. The expression of multiple opsins in the tail and MEX cells suggests a possible role in the regulation of the circadian rhythm in the skin. For all genes, RT-PCR analysis was conducted on 3 sets of biological replicates for the whole embryo and tail and 3 subculture sets for the MEX cells (N=3). E) Example of a pigmented melanophore.

Rho/rh1 , rhodopsin, lws, long wavelength sensitive, sws, short wavelength sensitive, VA-ops; vertebrate ancient opsin, pino, pinopsin

Conclusions

Preliminary data suggests that *opn4b*, the *Xenopus* homologue of the *melanopsin* gene, is differentially regulated in the eye and melanophores. In the eye, mRNA of *opn4b* appears to peak during the dark at zeitgeber (ZT-hour) 24 (dusk), while in melanophores *opn4b* mRNA peaks in the light phase at ZT 8. Visual opsins in the eye such as rhodopsin, and the cone Opsins (long- wavelength sensitive, and short-wavelength sensitive Opsins) do not appear to be regulated in a circadian manner . For the pineal opsin pinopsin is regulated in a circadian manner while VA opsin is not.

References

1. Sollars PJ, Pickard GE. The Neurobiology of Circadian Rhythms. Psychiatry Clin North Am. 2015 Dec; 38 (4):645-65