COMPARATIVE PHYSIOLOGY OF PERIPHERAL CLOCKS. CLOCK GENES (*Clock, Per*1, *Per*2, *Cry*1 AND *Bmal* 1) AND THEIR MODULATION BY LIGHT AND HORMONES IN FISH, AMPHIBIANS AND MAMMALS

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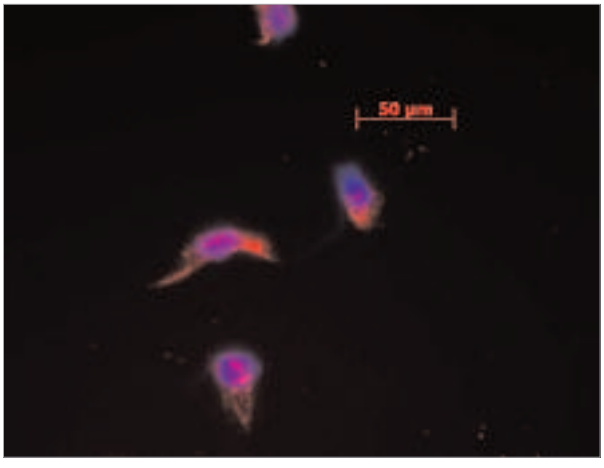

Immunolabeling of ZEM-2S embryonic cells with rabbit anti-serum UF061 (1:2,000) against Danio rerio *melanopsin (red, Cy3-labeled secondary antibody). In blue, nuclear staining with DAPI*

Melanopsin can be expressed, as well as other opsins, in skin pigment cells, to mediate photoresponses. Having in mind that cultured cells may constitute peripheral clocks and respond to visible light, we will analyze: 1. the mechanisms to adjust biological clocks in single cells of teleosts, amphibians and mammals; 2. their regulation by varying photoperiod regimes and hormones. We will then investigate: 1. whether the ZEM-2S embryonic cell line of *Danio rerio*, melanophores of *Xenopus laevis*, and B-16 F10 murine melanoma cells are able to cycle genes such as Per1, independently of light:dark cycles; 2. whether opsin expression is rhythmic, dependent on the integrity of the cellular clock and independent of the light:dark cycle; 3. whether opsin and clock gene expressions may be modified by hormones. These hypotheses will be tested by the quantification of luciferin bioluminescence originated from the activation of luciferase located in *Per*1 promoter, of mRNA (and of proteins whenever possible) of opsins and clock genes, under light:dark cycles, or constant darkness, in the presence of increasing hormone (melatonin, endothelin and α-MSH) concentrations, for increasing periods of time.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

To investigate the photosensitivity of ZEM-2S cells of the teleost *Danio rerio*, we accompanied the proliferation of cells maintained for 5 days on a regime of 14 hours of light by 10 hours of dark (14L:10D) and then transferred into constant darkness (DD), 14L:10D, 10L:14D or constant light (LL), which the rate of cell proliferation observed in cells submitted to constant light being lower. The expression of a photopigment is essential for this photo-sensibility and, in fact, we demonstrated the presence of RNA messenger for melanopsin (*Opn4x*) and for six *Crys* genes. The presence of the melanopsin protein was also demonstrated by immunocytochemistry. We then studied the temporal expression patterns of the genes *Per*1, *Cry*1*b*, *Clock* and *Opn4x* in ZEM-2S cells maintained for 5 days in 12L:12D or DD. In 12L:12D, the expression of *Opn4x* exhibited 2 peaks: at the start of the light phase and at the start of the dark phase. These peaks are also present in cells maintained in constant dark, during which the expression of *Opn4x* was significantly increased at all times, when compared to that observed in cells maintained in light: dark cycle. Although the expression of *Clock* does not vary over the 24 hour period, whether in 12L:12D or DD, it tends to increase during the dark phase and the subjective night, respectively. The clock genes *Per*1 and *Cry*1*b* exhibit robust circadian oscillation, with a significant increase 3h before the light phase, which persists during the entire photo-phase and declines abruptly in the dark phase. In constant dark, the amplitudes of temporal variation of *Per*1 and *Cry*1*b* attenuate, but the circadian rhythm remains significant. However, the peaks of expression appear shifted for the times of transition between subjective day and night. These results demonstrate that the ZEM-2S cells possess an intrinsic clock, since the rhythmicity of expression of the genes of the clock is maintained in constant conditions. Because such cells possess a functional photopigment, melanopsin, the adjustment of this clock can be effected by light. These cells, therefore, constitute an excellent model for the study of regulation mechanisms of peripheral clocks by light and hormones.

MAIN PUBLICATIONS

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