

Live Imaging of Circadian Gene Expression in Individual Fibroblasts using a Leica AS MDW Widefield Microscope

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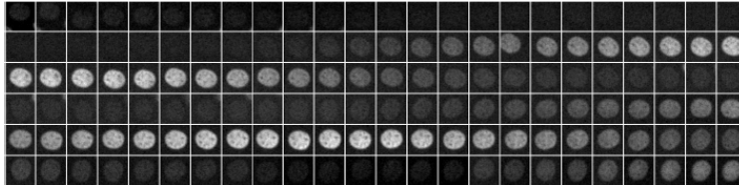
ABSTRACT:

The mammalian circadian timing system is composed of a central pacemaker in the suprachiasmatic nucleus (SCN) of the brain and subsidiary oscillators in most peripheral cell types. While oscillators in SCN neurons are known to function in a self-sustained fashion, peripheral oscillators have been thought to damp rapidly when disconnected from the control exerted by the SCN. Using two reporter systems, we monitored circadian gene expression in NIH3T3 mouse fibroblasts in real time and in individual cells.

To obtain meaningful and statistically relevant results cells were imaged and kept on the microscope stage for normally 72 hours at a time. This allowed following circadian gene expression over three period lengths. Being kept at 37°C in a humidified atmosphere containing 5% CO₂, cells did appear morphologically normal and were undergoing up to two full cell cycles.

Our results demonstrated that in vitro cultured fibroblasts harbor self-sustained and cell-autonomous circadian clocks and that circadian gene expression continues during cell division.

FIGURE:



Timelapse microscopy of circadian fluorescence in the nucleus of an individual cell after a serum shock.

Images were taken every 30 min during three consecutive days.

REFERENCE:

E. Nagoshi, C. Saini, C. Bauer, T. Laroche, F. Naef and Ueli Schibler, "Circadian Gene Expression in Individual Fibroblasts: Cell-Autonomous and Self-Sustained Oscillators Pass Time to Daughter Cells", *Cell*, **119**;5, 693-705 (2004)