LIGHT ENTRAINMENT OF SINGLE CELL CIRCADIAN OSCILATOR MEASURED BY A HIGH-THROUGHPUT MICROFLUIDIC DROPLET PLATFORM

Zhaojie Deng, Jonathan Arnold, and Leidong Mao*
The University of Georgia, USA

ABSTRACT

This paper reports the measurement on light entrainment of single cell circadian oscillator of a model fungal system, *Neurospora crassa* (*N. crassa*), through a high-throughput microfluidic droplet platform [1]. The results demonstrated for the first time that single cell circadian oscillators could be entrained by light.

KEYWORDS: Microfluidic droplet, Single cell analysis, Circadian rhythm, Light entrainment

INTRODUCTION

Light entrainment is one of the characteristics of a circadian/biological clock [2]. However measurement of light entrainment on single cells has never been conducted in a nonsynthetic system. This is because traditional measurement techniques (e.g., race tubes or microtiter plates) usually measure millions of cells for a population average. Therefore little is known about whether light entrainment is a feature of single cells, or an emergent property of a population of cells due to cell-cell communication. We previously developed a high-throughput microfluidic droplet platform that can measure circadian rhythms on a thousand *N. crassa* single cells [1]. Here we report using this platform to measure *N. crassa* single cells fluorescence intensities under 3 light entrainment conditions to investigate whether the single cell circadian oscillator of *N. crassa* can be entrained by light.

METHODS

The microfluidic droplet platform consists of a PDMS device for cell encapsulation and capillary tubing for storing droplets for long-term observation. An strain of *N. crassa* [3] was used to observe the transcriptional behavior of a *clock controlled gene*(*ccg-2*) through fluorescence imaging. The workflow of the platform is demonstrated in Figure 1. A LED light was used to control the light/dark (LD) cycles the cells experienced.

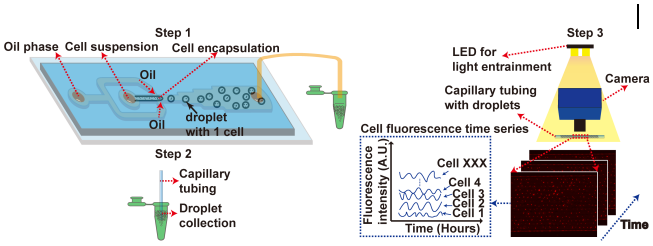

Figure 1: Workflow of the microfluidic droplet platform. Step 1: A droplet generation device for cell encapsulation. Step 2: Droplets are collected into capillary tubing. Step 3: Time-lapse fluorescence imaging by a CCD camera through a microscope under light entrainment and fluorescence data extraction.

RESULTS AND DISCUSSION

3 light entrainment conditions were used to study whether single cell circadian oscillator of *N. crassa* can be entrained by light. As a control, Figure 2(a) shows the fluorescent intensity of 1604 single cells under constant dark (blue curves) and an average intensity of all cells (red curve). No consistent oscillation was observed due in part to the heterogeneity of phases among single cells, however the average of periodograms of single cells showed a peak at ~21 hours in Figure 2(b), consistent with the period measured in previous study[3]. When introduced the 3 hours light/3 hours dark cycles, single cells are entrained to the LD cycles as shown in Figure 2(c): the average fluorescence intensity of 1330 single cells (red curve) is oscillatory. And in Figure 2(d), the average of periodograms of all the single cells shows a peak at ~6 hours. Also the single cell oscillator can be entrained to the 6 hours light/6 hour dark cycles as indicated in Figure 2(e): the average fluorescence intensity of 1626 single cells (red curve) shows oscillation. And the average of periodograms of all the single cells with a peak at ~12 hours in Figure 2(f) confirms that they oscillate with a period as the LD cycles. Single cells were also

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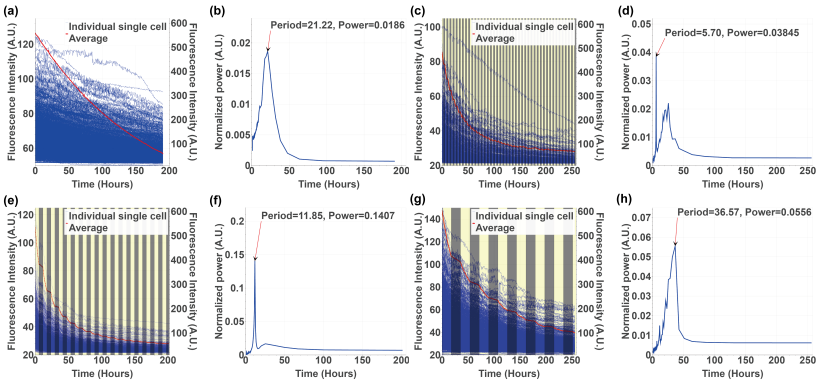

Figure 2: Single cell fluorescent intensities and average periodograms under different light entrainment conditions. (a) and (b) are results measured under constant dark condition: (a)1604 single cell fluorescent intensities (blue curves, right y axis) and the average of all the single cell fluorescent intensities (red curve, left y axis). (b)Average of the periodograms of all the single cells fluorescent time series. (c) and (d) are results measured under 3 hours light/3 hours dark: (c)1330 single cell fluorescent intensities (blue curves, right y axis) and the average of all the single cell fluorescent intensities (red curve, left y axis). (d)Average of the periodograms of all the single cells fluorescent time series. (e) and (f) are results measured under 6 hours light/6 hours dark: (e)1626 single cell fluorescent intensities (blue curves, right y axis) and the average of all the single cell fluorescent intensities (red curve, left y axis). (f)Average of the periodograms of all the single cells fluorescent time series. (g) and (h) are results measured under 18 hours light/18 hours dark: (g)1969 single cell fluorescent intensities (blue curves, right y axis) and the average of all the single cell fluorescent intensities (red curve, left y axis). (h)Average of the periodograms of all the single cells fluorescent time series.

exposed to 18 hours light/18 hours dark cycles and they follow the cycles of the 36 hours artificial day. As shown in Figure 2(h) and Figure 2(i), the average fluorescence intensity of 1969 cells is oscillatory and the periodogram shows a peak at ~36 hours. These results demonstrate that circadian oscillator of single cell of *N.crassa* can be entrained by light.

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CONCLUSION

The light entrainment property of single cell was studied here with the high-throughput microfluidic droplet platform. Large numbers of (> 1000) single cells were measured under different light entrainment conditions. And the results show that single cell circadian oscillator can be light entrained without any cooperation/communication with other cells. Therefore the circadian oscillator in single cells possesses the light entrainment property.

ACKNOWLEDGEMENTS

We acknowledge the financial support from National Science Foundation under Grant No. 1150042 and 1713746 and National Institutes of Health under Award No. R21GM104528,

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CONTACT

* Leidong Mao; phone: +1-706-542-1871; mao@uga.edu