Cell-free extract characterization

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Abstract

A cell-free extract system is an in vitro tool used to study biological reactions within cells. These extract systems reduce complex interactions found in whole cells by isolating subcellular fractions using ultracentrifugation. Cell-free extracts, particularly from *Xenopus* eggs, have been used in the discovery and characterization of cell-cycle regulators such as the protein complex cyclin B1-Cdk1 and the anaphase-promoting complex (APC). However, despite their utility, cell-free extracts have proven to have limitations, including quickly damped oscillations and differences in both dimension and spatial organization when compared to real cells. In identifying problems with the existing system, we developed an extract system encapsulating mixtures containing cycling *Xenopus* egg cytoplasm in cell-scale micro-emulsions. These droplet-based cells are stable and oscillate for dozens of cycles, offering advantages in tracking droplet activities. Despite improvements with the current system, we are interested in further describing our extract system based on specific characters observed in real cells. Previous work on *Xenopus* eggs has shown cell cycle periods to initially shorten with increasing division number followed by an increase in period at later divisions. To determine if this character is observed in our cell-free extract, we identified droplets exhibiting good oscillatory behavior and analyzed the correlation between average cycle period and the nucleus to cytoplasm ratio. We found that our extract system showed a continued increase in period as division number increased, a contrast to the trend observed in real cells.

1. Introduction

Cytoplasmic extracts from *Xenopus* eggs have played an important role in the discovery and characterization of central cell-cycle regulators. They have been used to investigate regulatory circuits within these extracts, assisting in understanding interlinked positive and negative feedback loops [3]. Extract systems are often preferable to in vivo systems, containing very specific recombinant molecules that are more suitable for systematic design, manipulation, and biochemical measurements.

In spite of these advantages, cell-free extract systems still pose major limitations. For instance, many extract systems generate oscillations that are quickly damped. Additionally, droplets often differ in dimension and spatial organization when compared to real cells. These limitations make it difficult to achieve cellular heterogeneity which is necessary to investigate important questions, such as stochasticity and tunability of the oscillator [5].

In an effort to amend problems with current extracts systems, we developed an artificial cell-free system by encapsulating reaction mixtures of cycling *Xenopus* egg cytoplasm in cell-scale micro-emulsions. Droplets from this system remain stable and continue to oscillate for dozens of cycles. This allows for improvements in long-term tracking of dynamic cycles within the droplet system [1].

We are motivated to further characterize this system, specifically as it pertains to observing how droplets from the extract compare to certain trends observed in real cells. Previous studies of embryonic cell division show cell cycle periods to initially shorten with increasing division number followed by an increase in period at later divisions. Figure 1 shows the average period of the cell shortened through division 6, began lengthening around division 9 and 10, and then increased in period at divisions 11 and 12 [2]. We are interested to see if analyzing the correlation between average period and the nucleus to cytoplasm ratio in the extracts will mimic trends observed in cells.

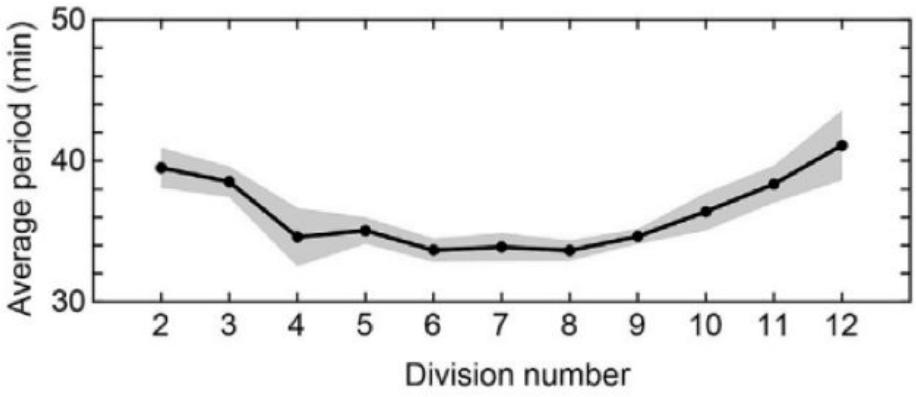

Figure 1: Average period versus division number for embryonic cells

To analyze this specific character of the droplets, we analyzed average cycle period in the extract droplets. We then plotted the average cycle time against the nucleus to cytoplasm ratio. We will consider the nucleus to cytoplasm ratio to be proportional to division number, as the nucleus to cytoplasm ratio increases with subsequent divisions. We then observed trends in the data for extracts from different days.

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2. Methods

2.1 Generation of droplet-based artificial cells

Xenopus extracts were prepared as described [4], but eggs were activated with 200 ng/μL calcium ionophore A23187 as opposed to electric shock. Extracts were kept on ice while applied with sperm chromatin (250 per μl of extract), GFP-NLS (10 μM), and recombinant mRNAs of securin-mCherry (10 ng/μL) and cyclin B1-YFP (ranging from 0 to 10 ng/μL). They were then mixed with surfactant oil 2% PFPE-PEG. A Fisher Scientific vortex mixer was used to mix 20 μl extract reaction mix and 200 μl 2% PFPE-PEG surfactant for 3 seconds at a speed of 10 to create droplets. Vibration speed and proportions of aqueous and oil phase were varied to obtain droplets over a range of sizes.

2.2 Image analysis and data processing

We used Imaris 8.1.2 for image processing. Level-set method on bright-field images was used for droplet segmentation and an autoregressive motion algorithm was used for tracking.

Data for the fluorescence intensities and areas of each droplet were collected for further analysis. Fluorescence intensity in droplets was normalized using the average intensity of the background. In order to calculate the period of each droplet cycle, Matlab was used to detect peaks and troughs over signal intensity for cyclin B-YFP and securin-mCherry [1]. It should be noted, that peak and trough analysis was limited to good droplets. Good droplets were identified as those which showed consecutive oscillations and limited background noise. Figures 2 and 3 show examples of good of bad droplet images, respectively, prior to selection.

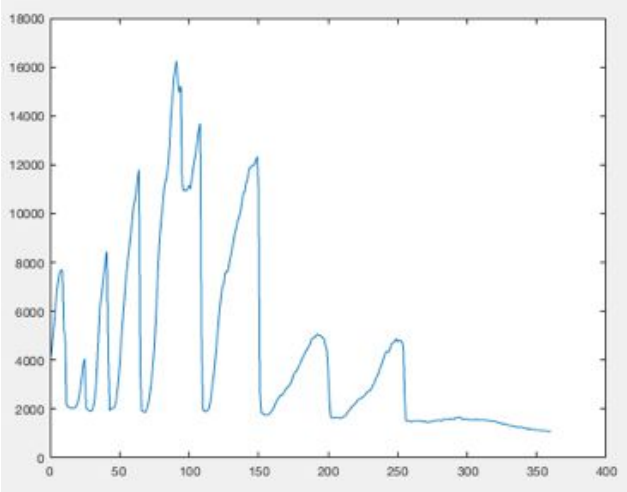

Figure 2: Matlab generated image of good droplet

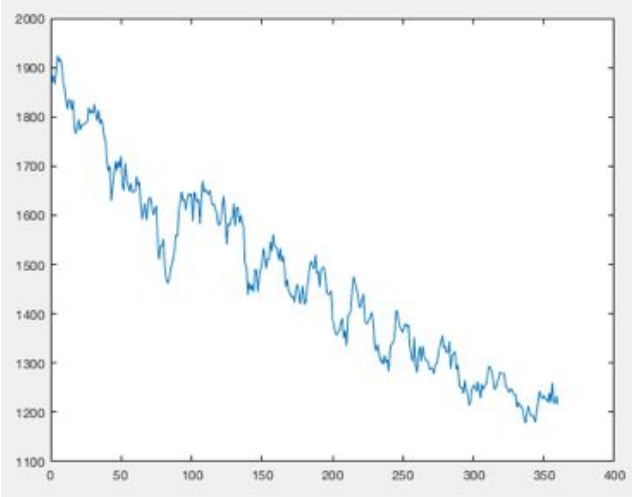

Figure 3: Matlab generated image of bad droplet

All peaks and corresponding troughs were manually edited to ensure correct selection. Each selected peak required selection of a corresponding trough on either side. The first peak was often left out of the selected data due to lack of a corresponding trough to the left of the peak.

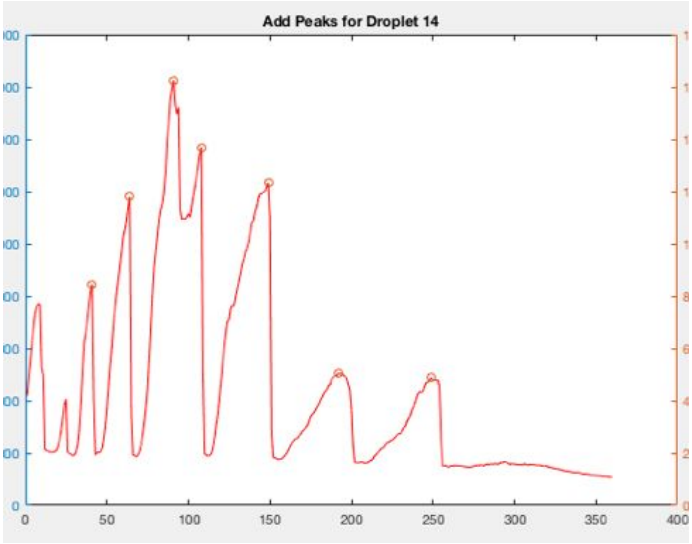

Figure 4: Peak selection for good droplet

Selected peaks and troughs corresponded to cycles within the droplets. To determine viable cycles we observed the segmentation for each frame. This was to ensure that there were existing nuclei that corresponded to each cycle. Clearly shown circular nucleus shapes in the segmentation indicated good cycles. Figure 4 shows the clear nucleus like shapes in segmentation characteristic of good cycles. Figure 5 on the other hand has no clear nucleus shapes. Both are included to provide examples of good and bad cycles.

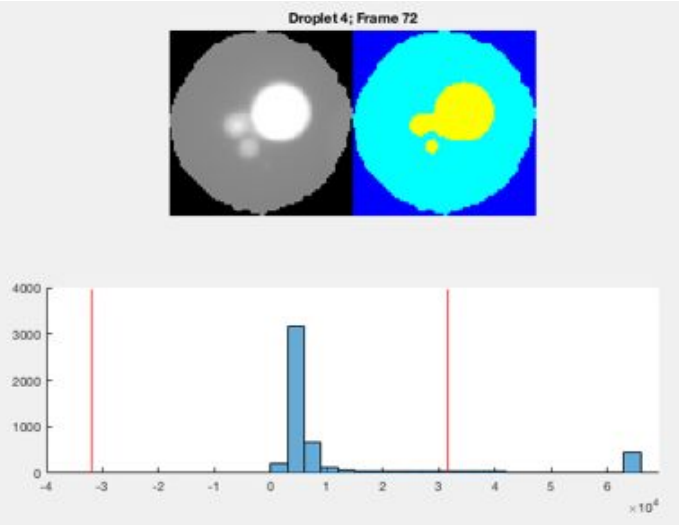

Figure 4: Segmentation for good droplet cycle

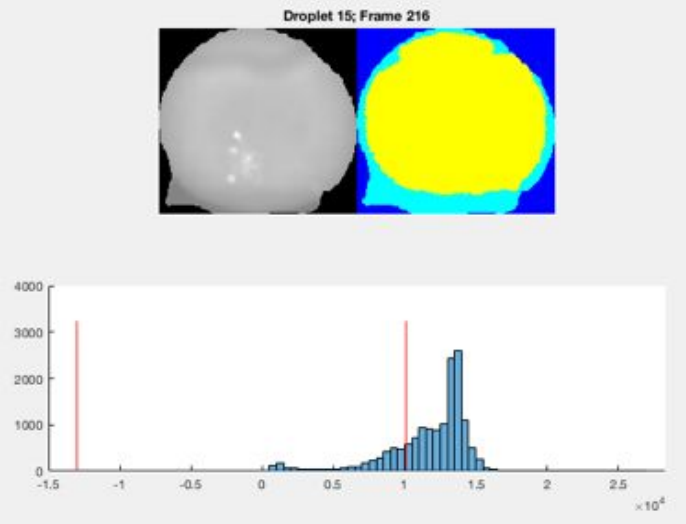

Figure 5: Segmentation for bad droplet cycle

Matlab was then used to calculate the average cycle times for each droplet and grouped to obtain a plot of average period versus nucleus to cytoplasm ratio for all chosen droplets in the cell-free extract. The data was then analyzed to observe trends for the extract droplets from different days.

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3. Results and Discussion

In examining the data from both days we identified an upward trend of increasing cycle period to nucleus to cytoplasm ratio. This is different from behavior observed in real cells in which cycle periods initially shorten, followed by an increase in period at later divisions. This trend was observed in data from cell-free extracts from two different days.

Figure 6 shows the trend for period over nucleus to cytoplasm ratio for the first day. It should be noted that the number of viable droplets varied between days. For this extract there were approximately 130 droplets with good cycles for the Matlab analysis. We observe a generally upward trend in data.

Figure 7 shows the same trend for an extract system from a different day. It should be noted that the number of viable droplets was significantly lower for this extract, only having approximately 30 droplets for analysis. Again we observe a generally upward trend, but with more variation in the data. This is most likely due to the limited size of useable data.

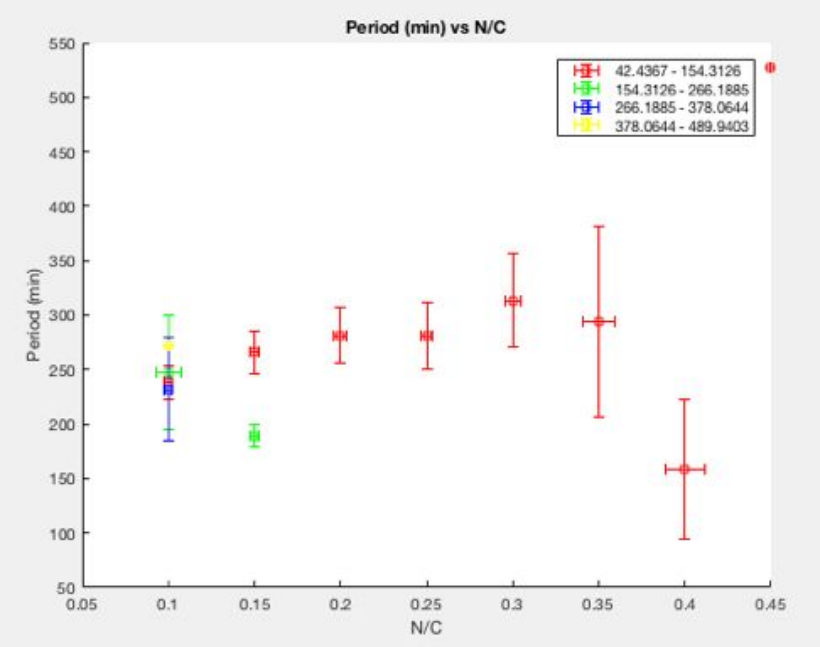

Figure 6: Period (min) versus N/C for Day 1 analysis

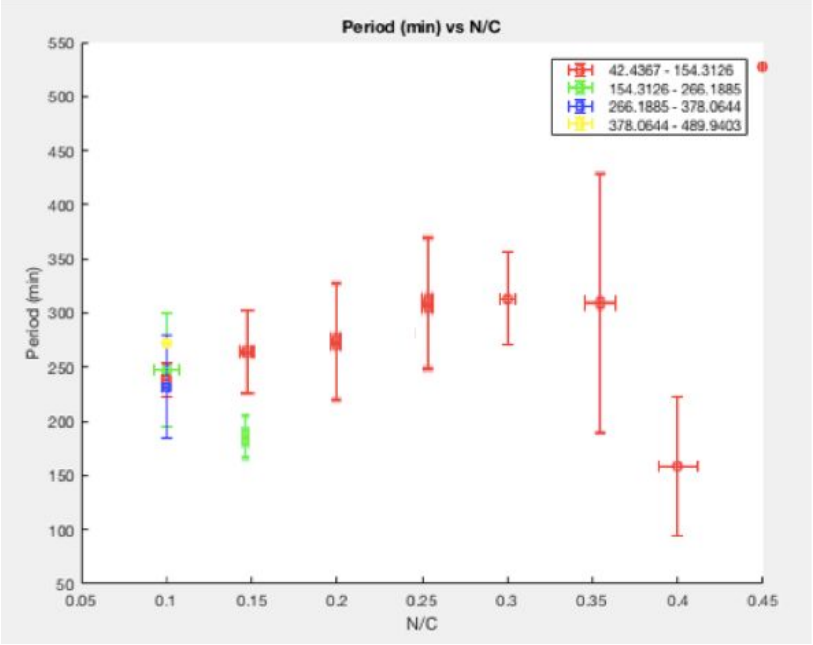

Figure 7: Period (min) versus N/C for Day 2 analysis

4. Future work

While current data suggests that our cell-free extract system does not exhibit the same behavior as real cells with respect to this character, it will be valuable to observe trends in the data for extract systems from different days with more viable droplets. As the number of number of viable droplets for both analyzed days was rather small, it will be important to observe trends for extract systems with larger sample sizes.

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