

Stochastic Gene Expression in Single Gene Oscillator Variants

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Abstract

It is infeasible to understand all dynamics in cell, but we can aim to understand the impact of design choices under our control. Here we consider a single gene oscillator as a case study to understand the influence of DNA copy number and repressor choice on the resulting dynamics. We first switch the repressor in the oscillator from the originally published lacI to treRL, a chimeric repressor with a lacI DNA binding domain that is inducible by trehalose. This slightly modified system produces faster and more regular oscillations than the original lacI oscillator. We then compare the treRL oscillator at three different DNA copy numbers. The period and amplitude of oscillations increases as the copy number is decreased. We cannot explain the change in period with differential equation models without changing delays or degradation rates. The correlation and phase coherence between daughter cells after cell division also tend to fall off faster

for the lower copy oscillator variants. These results suggest that lower copy number variants of our single gene oscillator produce more synchronized oscillations.

1 Introduction

Development of synthetic biological circuits requires design choices for circuit components and context. Often, circuit designs can be described at the level of interacting activators, repressors, and reporters, where there is a choice of which specific proteins to use in the circuit. The circuit is typically placed into a specific organism in a specific environment, with the circuit DNA either in the genome or on a specific plasmid. These design choices can affect circuit performance in a deterministic and stochastic sense.

Most circuits are designed with deterministic considerations in mind such as oscillators (1), toggle switches (2) and logic gates (3). However, single cells behave stochastically (4), and it is often of interest to suppress stochastic fluctuations (e.g. to get a more stable toggle or more regular oscillator). Design choices can impact both deterministic and stochastic circuit performance. In the case of an oscillator, the period and amplitude might change, but the coherence of oscillations between cells in the same lineage may also change.

Oscillators in general have a rich history of application to synthetic biology questions. The first synthetic circuit engineering dynamics in cells was an oscillator (1), and oscillators have also been used as examples to demonstrate other ideas such as in vitro prototyping (5), temperature compensation (6), and mitigation of loading effects (7). Notably, Veliz-Cuba et al. (8) use a synthetic oscillator to investigate the intrinsic and extrinsic components of gene expression noise. Here, we use a single gene oscillator as a case study to investigate the effects of plasmid copy number and repressor choice on circuit dynamics. The single gene oscillator is a good test circuit, because it is the simplest possible circuit that exhibits non-trivial dynamics.

In this work, we change the repressor as well as the DNA copy number in the single gene

oscillator and report the resulting differences in circuit behavior both in terms of the deterministic period and amplitude as well as the stochastic coherence of oscillations between sister cells. We attempt to explain the amplitude and period with the standard delay differential equation based modeling of the system (9), and we find that the amplitude variations are easily explained, but the period variations are not.

2 Results

2.1 Single Gene Oscillator Design and Implementation

Previously, Stricker et al.(10) developed the first single gene oscillator in *E. coli*. Their oscillator consisted of a single repressor lacI repressing its own production as well as the production of the fluorescent reporter yemGFP. When there is minimal lacI in the system, the system produces a lot of lacI and yemGFP. The level of lacI eventually builds up enough to repress production of lacI and yemGFP, and then lacI and yemGFP are degraded by the cell's native ClpX protease until the repression is relieved and another round of expression occurs. It can be shown that this system does not generate oscillations without a production delay in expressing the protein (9). However, we expect this delay to exist in general because of the inherent delays in transcription, translation, and protein folding and dimerization. Stricker et al. observed irregular oscillation periods in their system, where the cells' fluorescence did not always return to its baseline level in between expression peaks.

We built a similar single gene synthetic oscillator of our own. Our oscillator is a two plasmid system, where one plasmid contains the repressor gene and the other one contains the fluorescent reporter gene (Figure 1). The repressors we use are lacI and treRL, a chimeric repressor with the same DNA binding domain as lacI that has a ligand binding domain that responds to trehalose (11, 12). The versions of the lacI and treRL proteins that we use have a domain deletion that prevents tetramerization, and so we expect the repressors only to form dimers. The reporter is a very fast folding yellow fluorescent protein sfYFP (13). The

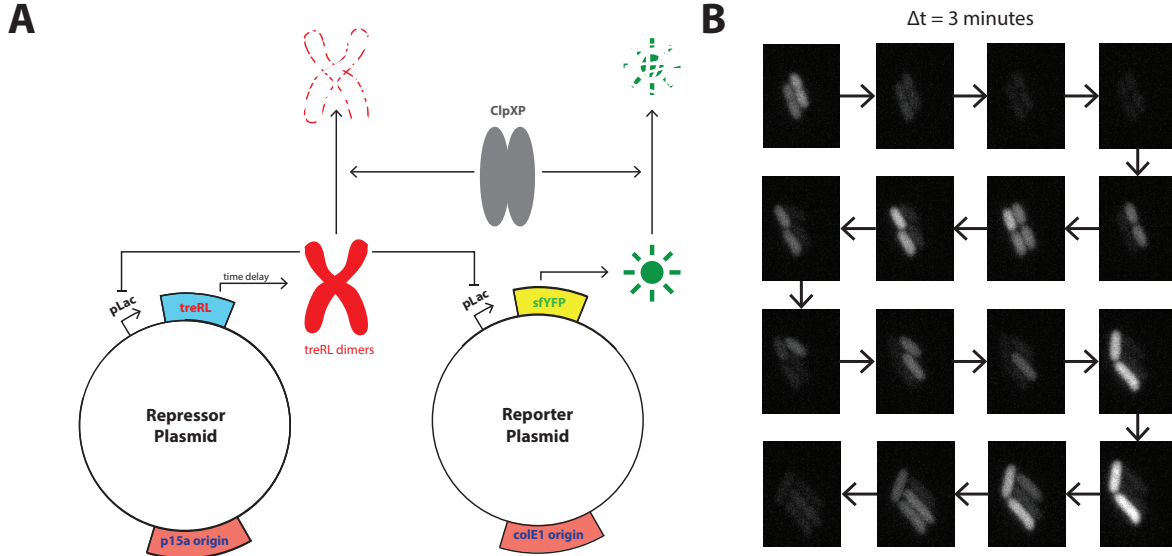


Figure 1: Circuit Background: (a) Circuit diagram of the treRL oscillator on p15a origin (b) Time lapse snapshots of treRL oscillator on p15a origin

repressor and reporter are both expressed on identical strong lac promoters with identical strong ribosome binding sites. Like the original single gene oscillator, our repressor and reporter are also tagged for active degradation by the ClpX protease.

The reporter plasmid has a colE1 origin, and so we expect there to be about 50-70 copies (14) of the reporter plasmid. The repressor plasmid can be on a medium copy p15a origin (20-30 copies) or a low copy pSC101 origin (10-12 copies). We also created a variant of the oscillator where the repressor gene is integrated into the genome in the phage 186 integrase site (15). We created a total of four oscillator variants: the lacI oscillator on a p15a origin, and the treRL oscillator on a p15a origin, on a psc101 origin, and integrated into the genome.

We imaged the oscillator variants on agarose gel pads using fluorescence microscopy at three minute intervals and utilized the Schnitzcell software (cite) to segment and track individual cell lineages.

2.2 Characterization of Oscillator Dependence on Repressor Choice

The p15a origin versions of the lacI and treRL oscillator were compared (Figure 2). The example trajectories of both oscillators were qualitatively similar in that all the cells oscillated with similar growth rates (Figure 5). However, one major qualitative difference was that the lacI oscillator sometimes did not return to zero fluorescence between expression peaks (Figure 2A). This finding is consistent with the results of Stricker et al.(10).

Quantitatively, the oscillations differed considerably. The lacI oscillator exhibited a significantly larger period and amplitude than the treRL oscillator. The treRL oscillator also outperformed the lacI oscillator in a stochastic sense. The treRL oscillator maintained a higher correlation between daughter cells after cell division for about 25 minutes before dropping below the lacI oscillator. The treRL oscillator also maintained less phase difference between daughter cells after cell division. The error bars on the correlation plot are one standard deviation generated by bootstrapping, while the error bands on the phase difference plot are standard error of the mean. It is important to note that the time scale is not normalized to the oscillator period or the cell division time in these plots. Thus, this means that the treRL oscillator exhibits less phase decoherence after almost two periods than the lacI oscillator does after just one period.

2.3 Characterization of Oscillator Dependence on DNA Copy Number

Next, we compared the treRL oscillator at different copy numbers. First, we swapped out the p15a replication origin for a psc101 replication origin keeping the directionality of the replication origin and the rest of the plasmid the same. We also integrated the treRL gene with terminator into phage 186 integrase site in the genome (15).

We found that the period and amplitude of fluorescence oscillations increased as the DNA copy number decreased. The p15a oscillator notably had a mean period of 16.4 minutes,

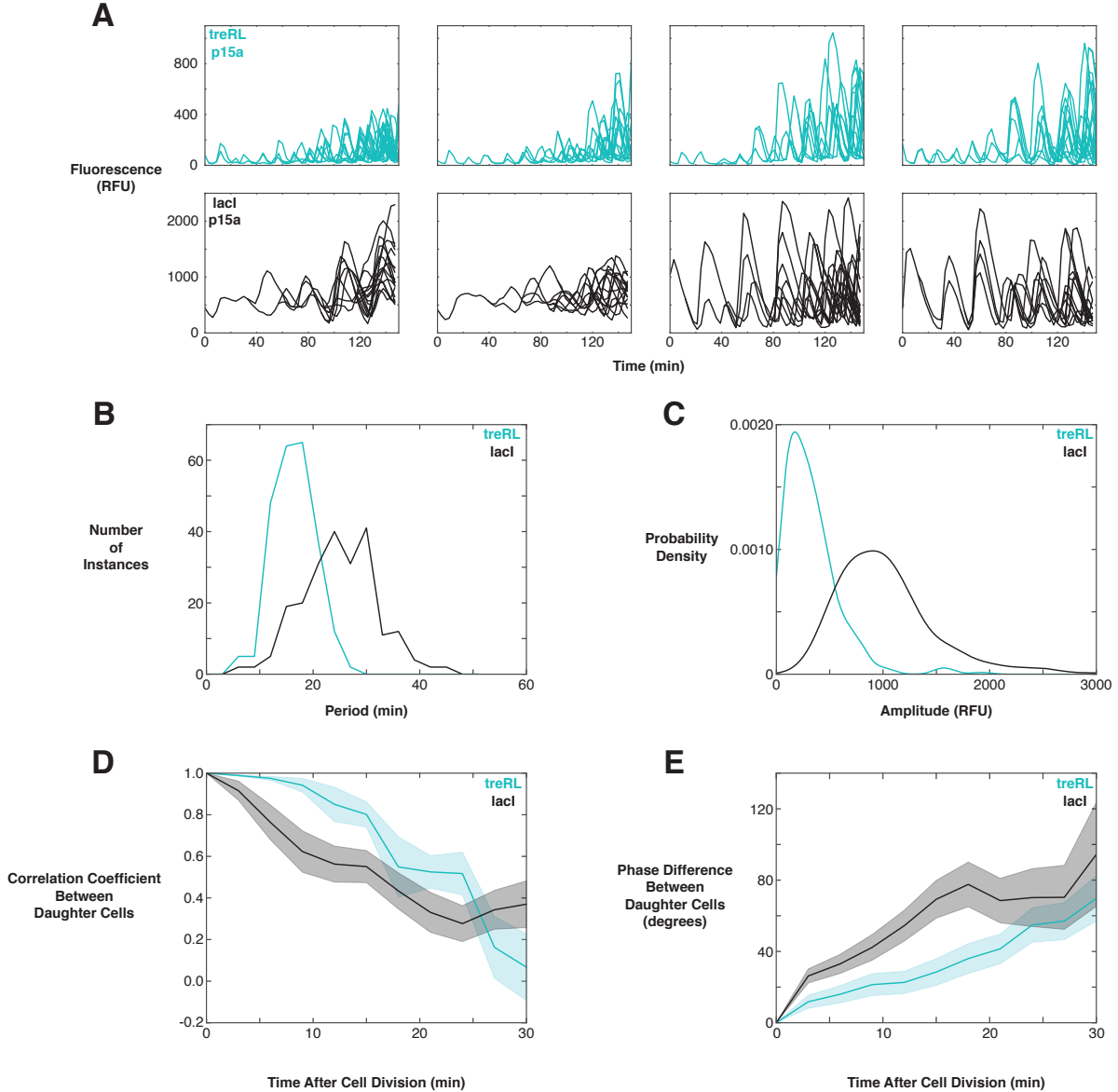


Figure 2: treRL and lacI comparison: (a) Example fluorescence trajectories for both the treRL and lacI oscillators (b) Distribution of oscillation periods for treRL and lacI (c) Distribution of oscillation amplitudes for treRL and lacI (d) Correlation in fluorescence between daughter cells after cell division. treRL stays higher initially and then decays. Error bars are bootstrapped one standard deviation.

which we believe is the fastest synthetic genetic oscillator to date. The single copy treRL oscillator had a mean period of 28.9 minutes similar to the 24.9 minute mean period of the lacI oscillator on a p15a origin. Additionally, the amplitude distributions widened for the lower copy oscillators. The period distributions were also wider for the lower copy oscillators.

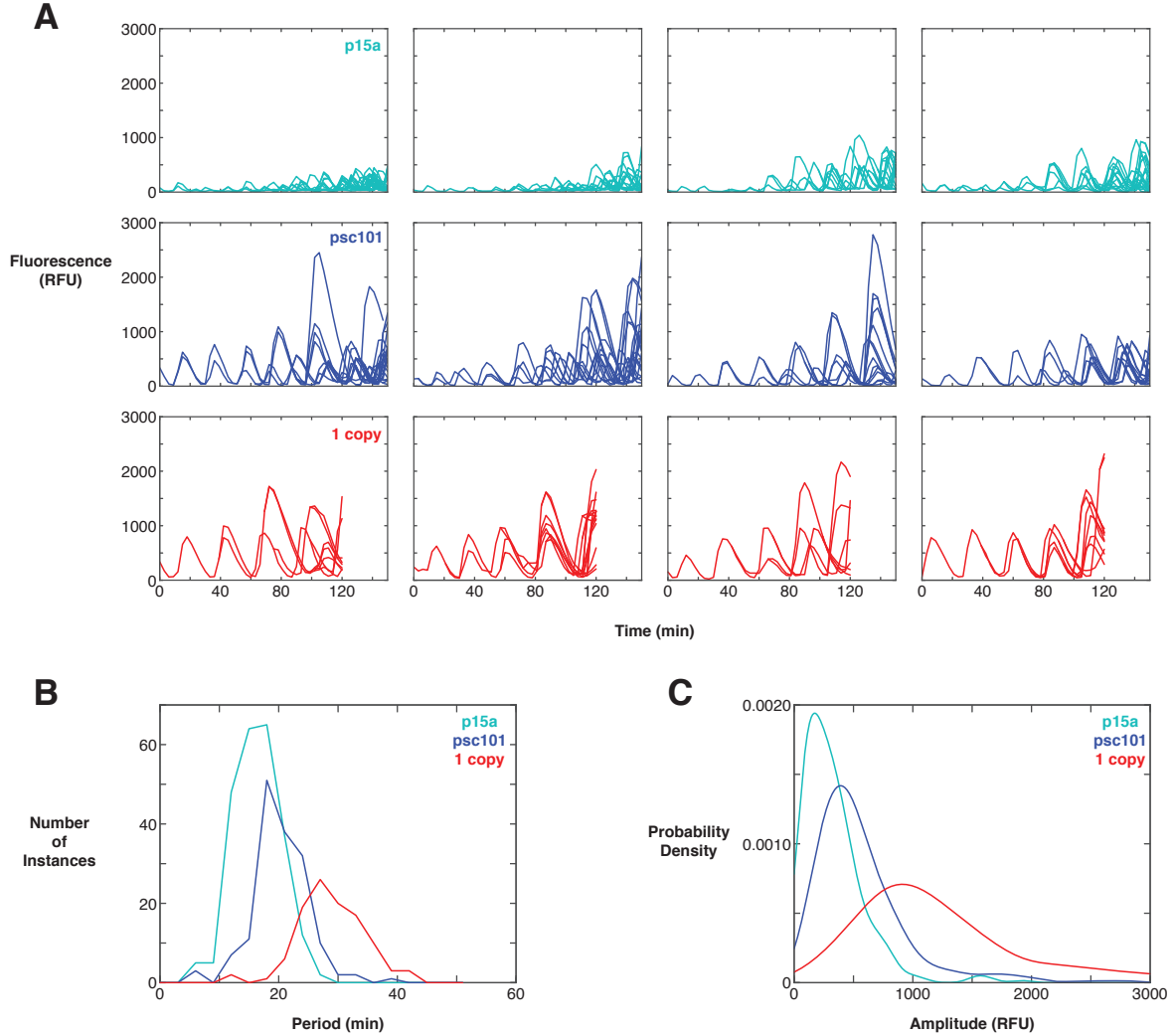


Figure 3: treRL copy number variants: (a) Example trajectories of treRL oscillator on p15a, on psc101, and at single copy (b) Period distribution for each copy number variant of treRL oscillator. Lower copy variants have longer periods. (c) Amplitude distribution for each copy number variant of treRL oscillator. Lower copy variants have bigger amplitudes.

We also computed the phase and correlation decoherence for the oscillator at different copy numbers (see Figure 4). For correlations, we found that the lower copy psc101 and single copy oscillator stay fairly correlated for 30 minutes after cell division, while the p15a oscillator correlation drops precipitously after about 15 minutes. Of course, this corresponds to about one period for the treRL oscillator, and so the behavior may be explainable by some period based phenomenon.

We found that the phase decoherence has no clear trend with respect to the copy number.

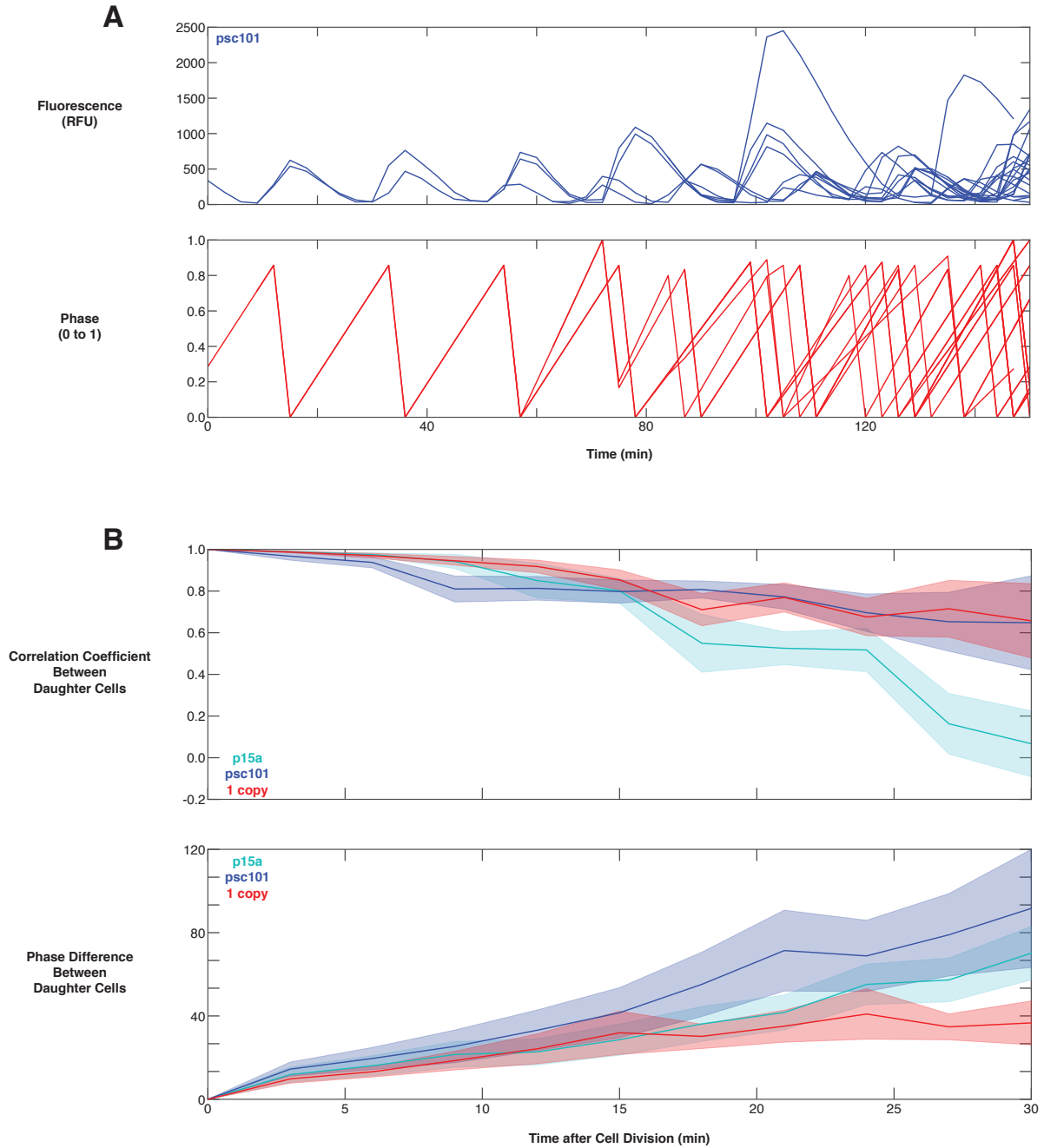


Figure 4: Phase and Correlation Decoherence: (a) Example of phase plotted along with an example trajectory lineage (b) Average phase difference between daughter cells grows as time after cell division increases. Phase decoheres more slowly for lower copy number variants. Error bands are s.e.m. (c) Correlation between daughter cells decays as time after cell division increases. Lower copy number variants stay more correlated over time.

There is no statistically significant difference in phase decoherence between the psc101 and p15a oscillators. However, the single copy oscillator exhibits better phase coherence than the

psc101 oscillator at 18 minutes or greater after cell division and better coherence than the p15a oscillator at 27 minutes or greater after cell division. The increase in phase decoherence with time is also much flatter for the single copy oscillator than the other two oscillators.

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3 Supplementary Information

The cell cycle time distributions were computed for each of the oscillator variants and reported in Figure 5. Because cell division time sets the dilution rate for cells, it is important that the cell division time distributions are similar between experiments. This gives more confidence in ascribing differences in oscillator dynamics to the inherent differences in the repressor and DNA copy number. The cell division times are similar between three of the experiments, but cells take longer to divide in the treRL p15a oscillator. This effect is notable because despite the lengthier cell division time, the treRL p15A oscillator still exhibits the fastest period.

The mean and standard deviation of the cell division times are included in Table 1.

Table 1: Cell Division Time Statistics

| Experiment | Cell Division Time mean \pm std (minutes) |
|-------------------|---|
| lacI p15a | 32.0 ± 7.4 |
| treRL p15a | 36.2 ± 8.5 |
| treRL psc101 | 34.5 ± 6.9 |
| treRL single copy | 33.3 ± 6.8 |

The actual statistics for the period and amplitude are reported below in Table 2.

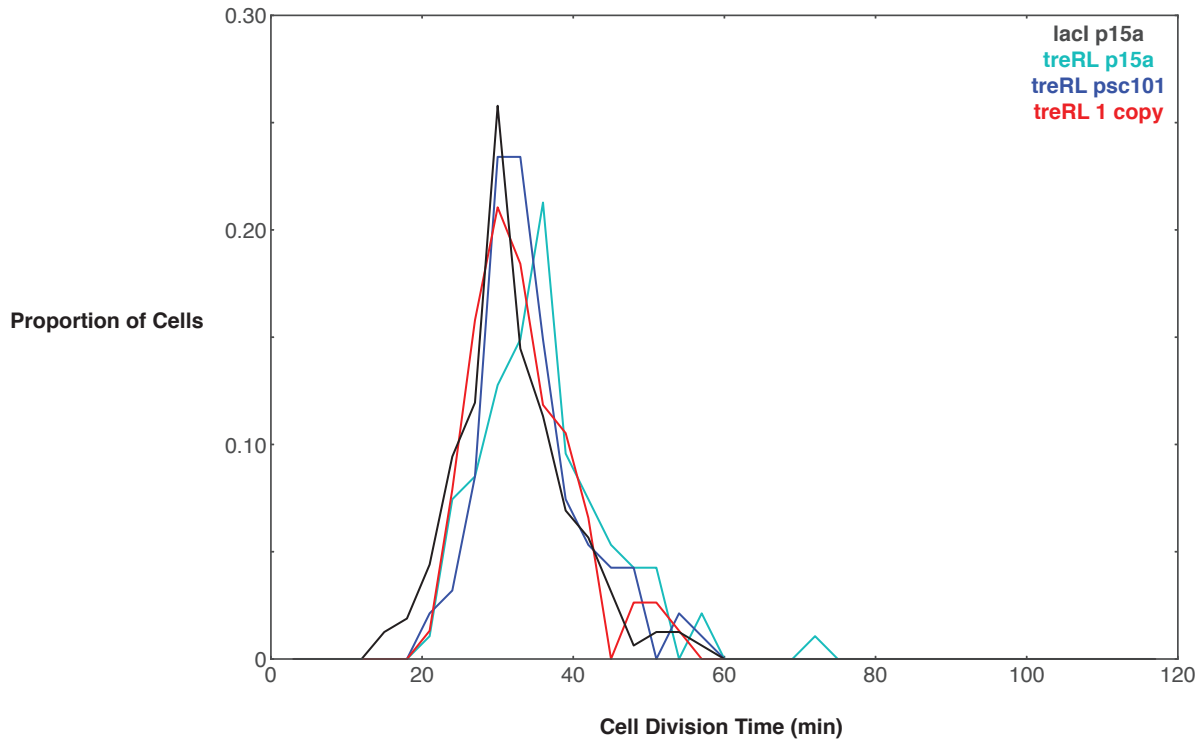


Figure 5: Cell Division Times Across Experiments

Table 2: Period and Amplitude Statistics

| Experiment | Period mean \pm std (minutes) | Amplitude mean \pm std (RFU) |
|-------------------|---------------------------------|--------------------------------|
| lacI p15a | 24.9 ± 7.1 | 1022 ± 452 |
| treRL p15a | 16.4 ± 3.9 | 327 ± 265 |
| treRL psc101 | 20.3 ± 4.7 | 559 ± 397 |
| treRL single copy | 28.9 ± 5.5 | 1213 ± 689 |

The detailed plots of the distributions of daughter cell fluorescence pairs as well as daughter cell phase differences after cell division are included below in Figure 6 and Figure 7.

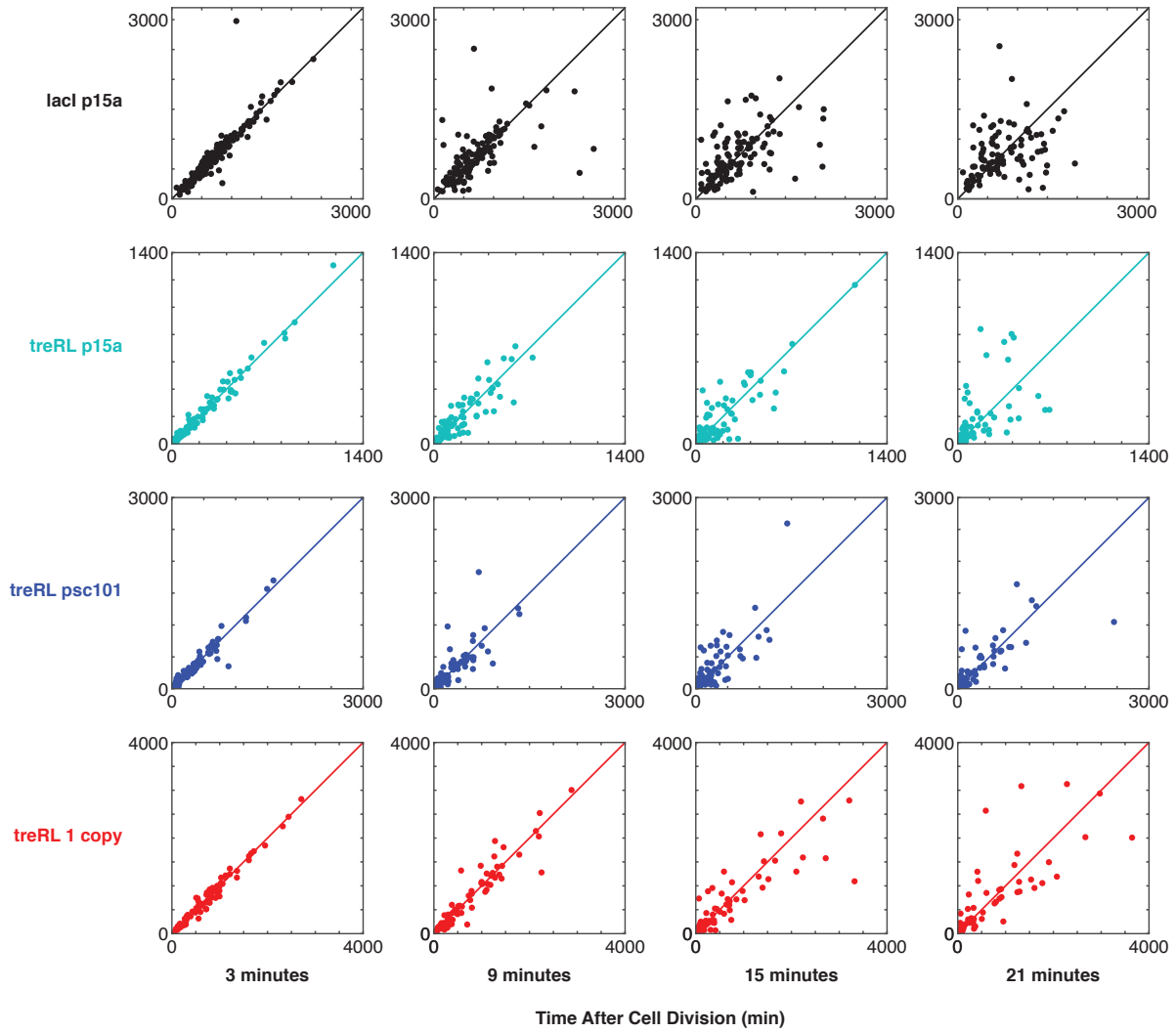


Figure 6: Daughter cell fluorescence scatter plots at varying times after cell division: The plots are initially highly correlated and become less correlated as time after cell division increases. The solid line in each plot is the line $y = x$ signifying the behavior of a truly deterministic system.

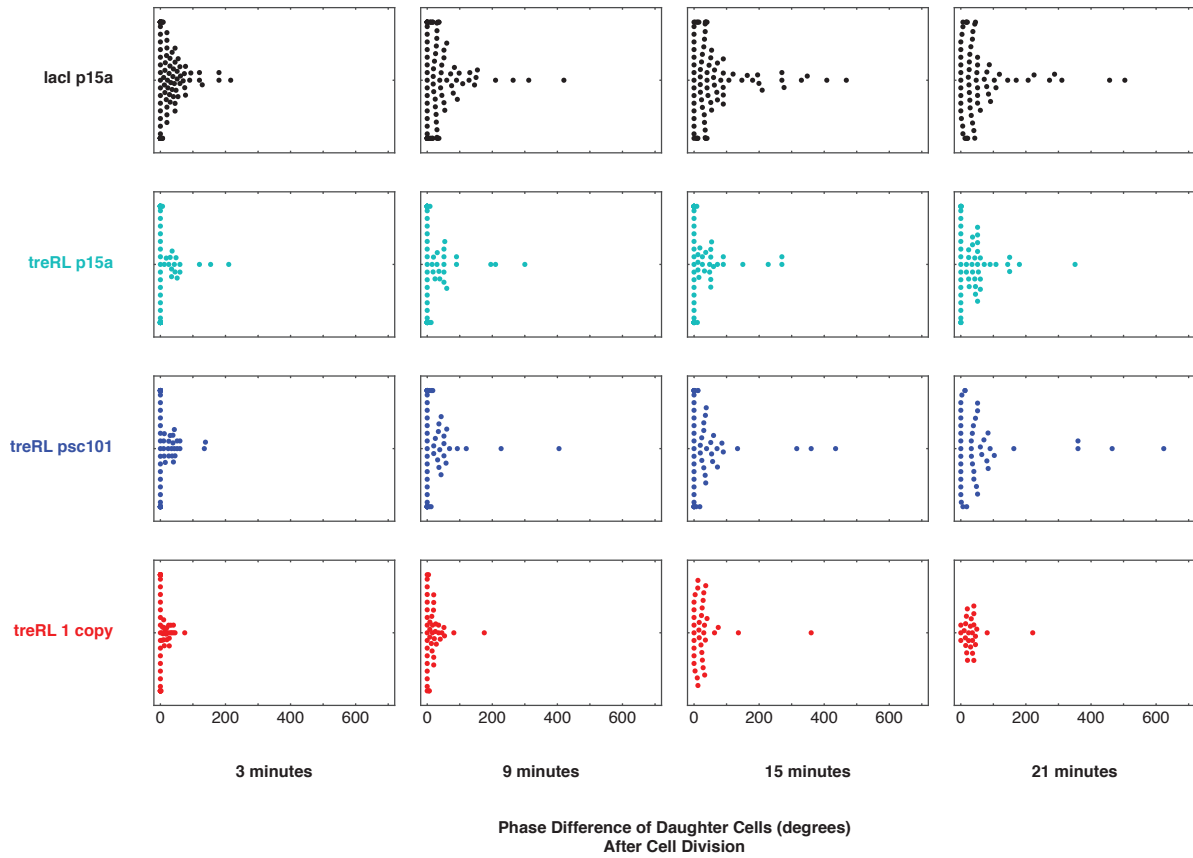


Figure 7: Daughter cell phase difference at varying times after cell division: Each plot is a swarm plot depicting the phase difference distribution for the given time and oscillator variant.

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