

SI1: Synchronization and interdivision time

Ulrik de Lichtenberg¹†, Rasmus Wernersson¹†, Thomas S. Jensen¹†, Henrik Bjørn Nielsen¹, Anders Fausbøll¹, Peer Schmidt², Flemming Bryde Hansen¹, Steen Knudsen¹ and Søren Brunak^{1*}

†These authors contributed equally to this work ¹Center for Biological Sequence Analysis, BioCentrum, Technical University of Denmark, Building 208, DK-2800 Lyngby, Denmark. ²febit ag, Käfertaler Strasse 190, D-68167 Mannheim, Germany *Corresponding author: Tel: (+45) 45 25 24 77, Fax: (+45) 45 93 15 85, email: brunak@cbs.dtu.dk

In this supplementary information, we address the issue of cell cycle synchrony from a number of different angles and estimate the interdivision time. We find that the culture appears clearly synchronized in both the first and the second cycle and find close agreement between two independent estimates of the interdivision time.

Monitoring synchrony

As described in Materials and methods, the synchronized yeast culture was grown in a fermentor for 300 minutes with samples taken every 20 minutes. Synchronization was obtained through arrest-and-release of the temperature sensitive mutant strain *CDC15-2* (Spellman *et al.*, 1998; Futcher, 1999). The culture was grown to a density of $OD_{600} = 0.8$, and the temperature was raised slowly to 37°C over 3 hours (1.5°C/15min for the first 2 hours and 0.5°C/15min for the last hour) and kept at 37°C for 1 hour. At this point, phase-contrast microscopy confirmed that virtually all cells had the dumbbell shape characteristic for arrested *cdc15-2* mutants (see Figure 1), described by Futcher (1999).

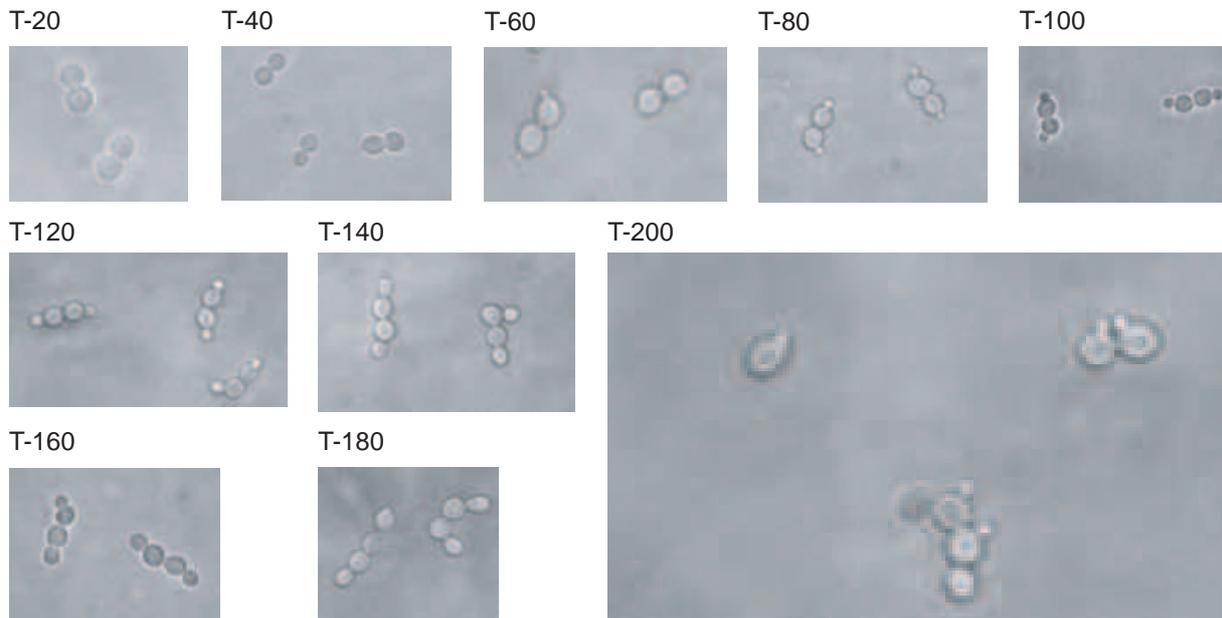


Figure 1: Phase contrast microscopy images. Selected images, showing examples of the cell morphology at different stages during the experiment. The first small buds appeared at 40-60 min and the next generation of buds was seen at 180-200 min.

Throughout the experiment, the synchrony of the culture was monitored by phase contrast microscopy (Figure 1), and the growth rate of the culture through measurements of OD_{600} and CO_2 efflux.

Estimating the interdivision time

Based on the microscopy results, we counted the fractions of cells with i) No visible buds ii) small buds, size $<1/3$ iii) medium buds, size $1/3-2/3$ and iii) large buds, size $>2/3$ — all sizes relative to the mother cell — at every time point. Randomly selected cells from phase contrast microscopy pictures (40x magnification) were used.

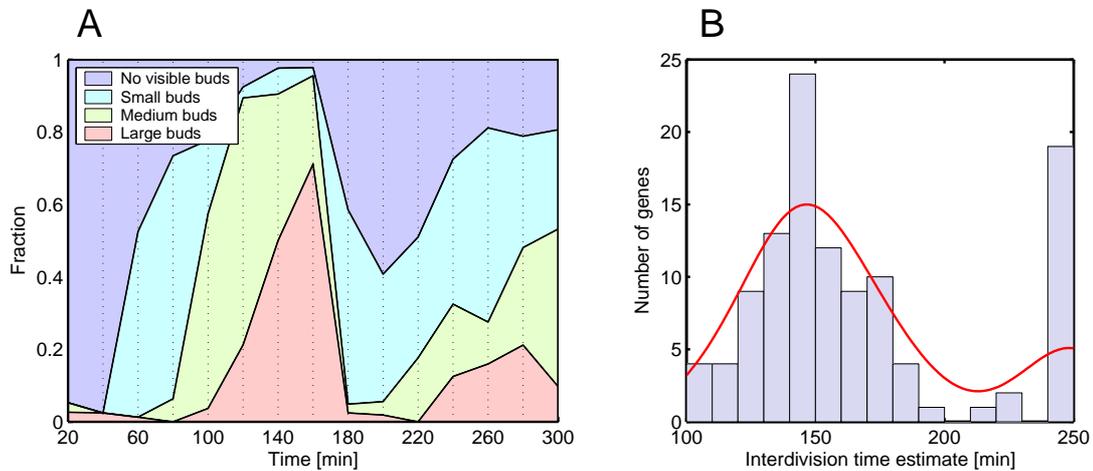


Figure 2: Estimation of interdivision time. **A)** Fraction of cells with i) No visible buds ii) small buds iii) medium buds and iii) large buds (see Materials and methods). **B)** Histogram and smoothed distribution (gauss kernel density estimation) of optimal interdivision times for 113 known cell cycle regulated genes (Johansson *et al.*, 2003). The distribution shows a peak at 148 minutes, in good agreement with the bud-counting estimate. The peak at the extreme end of the scale is caused by genes that do not appear periodic in this experiment.

Figure 2A summarizes the results and indicates synchrony in both cycles. The synchronization method releases cells from arrest in late M phase (Yeong *et al.*, 2000), and we see the first buds appearing between the 40 min and 60 min time points. The culture appears clearly synchronized in the first cycle and a new generation of buds appears at 180-200 min, also indicating synchrony into the next cycle. The microscopy data thus displays an interdivision time around 140 min. However, due to the particular morphology of arrested *CDC15-2* cells, distinguishing the categories becomes difficult in the second cell cycle: During the arrest, the cell wall becomes fused in the original mother/daughter cell pair, which leads to the characteristic dumbbell shape (Futcher, 1999). In the second cell cycle, the culture is a mix of the original fused cells and single cells (detached buds from the first cell cycle), making it difficult to compare the second cycle to the first. In particular, it is virtually impossible to distinguish between “no buds” and “large buds”. See Futcher (1999) for a detailed discussion of the *CDC15-2* strain.

Orthogonal to the interdivision time estimate based on microscopy, we used the microarray data directly to estimate the period that contained the maximum signal. A reference set of 113 genes (Johansson *et al.*, 2003) (cell cycle regulated in small scale experiments) was used and for each of these, the period that gave the maximum Fourier score (see Materials and methods) was determined. Figure 2B shows the distribution of these “optimal periods”. A clear peak was seen at 148 min, in good agreement with the conclusion of the microscopy analysis (140 min). The second, smaller peak at the extreme of the scale (250 min) was an artifact caused by genes that (in this experiment) exhibit an almost constant, non-cyclic pattern of expression and thus correlate best with the longest possible period.

A final check for synchrony

For the total set of 397 genes identified as periodic (see the main paper), the time of peak expression was estimated (see Materials and methods). This *peak time* is given as a fraction of the cell cycle completed, with time zero defined as the time of cell division (de Lichtenberg *et al.*, 2005).

Figure 3 relates the characteristic cell cycle phases to this timescale by showing the expression of five phase specific gene clusters defined by Spellman *et al.* (1998). It demonstrates that the phases occur in the correct consecutive order in this experiment, as would be expected in a synchronized cell culture. The

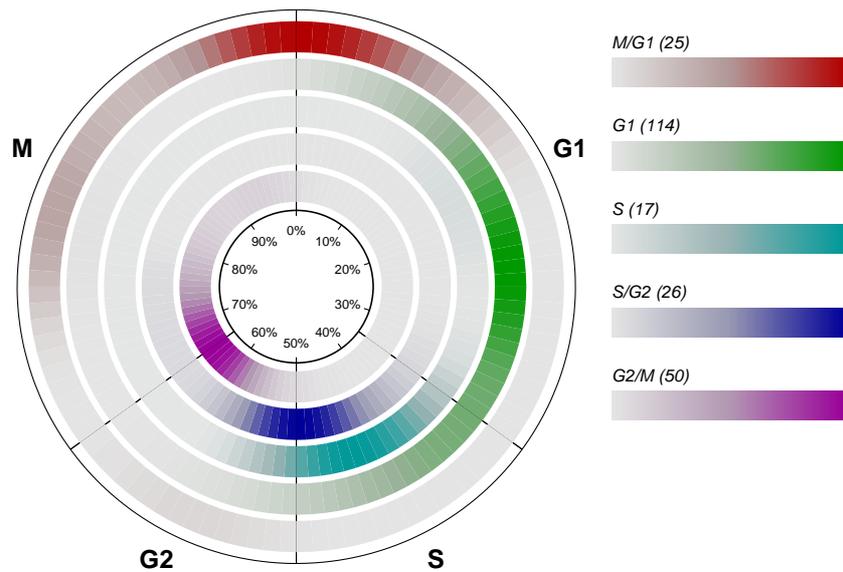


Figure 3: Phase specific gene clusters. A peak time was estimated for each periodic gene, describing where in the cycle the gene is maximally expressed (see Materials and methods). Time is given as a fraction of the cycle completed, with zero defined as the time of cell division. For each of the five gene clusters defined earlier by Spellman *et al.* (1998), we selected the genes that scored above the 20% FDR threshold in this experiment (with the number of genes in each cluster in parentheses). The circles show the smoothed distribution of peak times for these genes in each cluster. Zero time was set at the peak of the M/G₁ cluster. Based on the time of expression of these five clusters, the positioning of the four characteristic phases (G₁, S, G₂ and M) was estimated, as illustrated by the outermost black circle.

Figure was used to estimate the phase in which the periodic genes peak, as illustrated by the outermost black circle.

Homology

All yeast Open Reading Frames (ORFs) were blasted against a set of 1134 human cloneIDs identified as periodically expressed in human HeLa cells by (Whitfield *et al.*, 2002). CloneIDs with a e-value lower than 10^{-5} were considered homologs. *S. pombe* orthologs were provided by Valerie Wood at the Sanger Centre.

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