SUPPLEMENTARY

1. Quantitative Facs analysis: From BrdU measured fraction of replicated DNA to Facs histograms

The fraction of replicated DNA, $f_{DNA}(t)$, was calculated by measuring the kinetics of BrdU incorporation into DNA (Fig.2A). We used the dynamical method developed by Bertuzzi et al (1) to analyse the Facs histograms. To show that both methods are consistent and complementary we reproduce the Facs profile of an exponentially growing cell population from the measured $f_{DNA}(t)$ using the Bertuzzi's method. It is important to keep in mind that the profile of Facs histograms in a synchronized cell population depends on two parameters: the DNA content distribution of cells at a given time and the total rate of DNA synthesis. However, in the case of an exponentially growing cell population the Facs profile depends solely on the rate of DNA synthesis (1, 2). Thus, if the used analytical methodology is correct the knowledge of $f_{DNA}(t)$ should be enough to reproduce the Facs profile of an exponentially growing cell population. During our experiment, in order to identify the G1 and G2/M peaks in the Facs histograms, a fraction of the sample was grown exponentially in presence of 100μM thymidine at 30°C in synthetic minimal media supplemented with adenine and casamino acids (2%). Using this fraction we followed the exponential growth of the S. cerevisiae cells by measuring the turbidity of the solution at 600 nm every 15 min over 6 hours. By fitting the obtained data to an exponential we measured the Malthusien growth exponent of our strain as $\alpha = 5 \times 10^{-3} \pm 1 \times 10^{-3} \mathrm{min}^{-1}$. The obtained value is compatible with the doubling time of 2.5 h reported by Langronne et al (3) for this yeast strain. A fraction of the sample containing 10^4 cells was characterised by Facs. Bertuzzi et al (1) developed an equation linking the Facs profile and the total rate of DNA synthesis for an exponentially

grown cell population:

$$\tilde{\nu}(\xi) = 2K(\xi,1) - 2K(\xi,2) + (2-\vartheta_1)\int_1^2 \frac{\delta K(\xi,x)}{\delta x} e^{-\int_1^2 \frac{dz}{\omega(z)}} dx \qquad (1)$$

$$K(\xi,x) = \frac{1}{\sqrt{2\pi}\sigma(x)} e^{-\frac{(\xi-x)^2}{2\sigma(x)^2}} \qquad (2)$$

$$\sigma(x) = C\sqrt{x} \qquad (3)$$

where $\tilde{\nu}(\xi)$ is the normalised fluorescence density of the detection channel ξ normalised by the position of the G1 peak. $K(\xi, x)$ is the kernel function representing the dispersion

of PI fluorescence over the detection channels for a cell whose DNA content is x. The form of $\sigma(x)$ was proposed by Bruni et al. (4) on the basis of a model of DNA-dye interaction. The parameter C corresponds to the width of the G1 peak ($x = 1$) and is measured to be $C = 0.24 \pm 1.3 \times 10^{-3}$. The parameter θ_1 represents the fraction of cells in G1 and represents a fraction of 0.2 of the total population. The function $\omega(z)$ is equal to $\omega(1 + f_{DNA}(t)) = \frac{1}{\alpha}\frac{df_{DNA}(t)}{dt}$, where $\frac{df_{DNA}(t)}{dt}$ represents the rate of total DNA synthesis in a cell with $z = 1+f_{DNA}(t)$ DNA content. Using the rate of total DNA synthesis calculated from the fraction of replicated DNA (Fig. 2B) we calculate the Facs profile of exponentially grown population (Figure S.1) and compare it with the experimental measurements. Both curves are in good agreement. Therefore the two used methods are consistent and equivalent and so the cell density $n(t)$ extracted from Facs data can be used to analyse the rate of origin firing in our experiments.

2. Are the measured *I(t)* consistent with the replication fraction that can be inferred from the data?

We have extracted from published microarray data and combed DNA fibres the rate of origin firing $I(t)$. In order to check the validity of our approach we should be able to calculate the

fraction of replicated DNA from the extracted *I(t)* profile. In their kinetic modelling of DNA replication Bechhoefer et al (reviewed in (5)) used the formal analogy between DNA replication and one dimensional crystallization kinetics to link the rate of origin firing to the fraction of replicated DNA:

$$f_{DNA}(t) = 1 - e^{-\left(2v\int_0^t\int_0^{t'} I(t'')dt''dt'\right)} \qquad (4)$$

where v is the speed of progression of replication fork and is set to v = 1.46 kb.min^{-1} (6). Therefore the unique knowledge of the temporal profile of *I(t)* is enough to calculate $f_{DNA}(t)$. Fig. S2 represents the calculated $f_{DNA}(t)$ using equation 4 and the extract $f_{DNA}(t)$. As it can be observed the two $f_{DNA}(t)$ are in good accordance. Therefore, the extract form of *I(t)* is consistent with the extracted form of $f_{DNA}(t)$.

3. Effect of eye size on the relation between *I(t)* and $N_f(t)$

To extract the *I(t)* profile from both microarray and combing experiments we only counted eyes with sizes smaller or equal to the experimental resolution. The $N_f(t)$ profile was determined by considering only boundaries on the fibres in the case of combing experiments, and on the horizontal cuts in the case of microarray data. However, this methodology has an unavoidable bias that underestimate the number of fired origins and the number of replication forks. This effect should be particularly strong at the end of S phase where the size of unreplicated regions decrease and therefore the detection of a newly fired replication origin close to a replication fork become difficult.

As we measure that the *I(t)* and $N_f(t)$ are proportional even at the end of S phase, we should check whether this proportionality depends on the described bias or not. To address this issue, we ran a set of simulations as described previously (7) for a hypothetic genome of length equal to 1000 kb. At each calculation round we measured the rate of origin firing and the number of replication forks assuming that the origin detection and boundaries detection suffers from a resolution of Δx. We ran 2 sets of 1000 simulations, the first set corresponds to

a random origin firing process with a probability P = 1×10^{-4} and the second sets assumes that the probability of origin firing is a function of fork density, $P = 3\times10^{-4} + 2\times10^{-3}\left[1 - e^{-\frac{N_{fork}}{140}}\right]$.

Fig S3 show the average *I(t)* and N_f *(t)* for both situations for different values of Δx. These results show that the linear relation between the rate of origin firing and the fork density is independent of Δx and is only observed in the case where the rate of origin firing is a function of the fork density.

4. Remarks on the assumption of an "uniform law of cell-DNA synthesis over the population".

To propose a method that reproduces the Facs profiles of a growing cell population Bertuzzi et al (1) considered 3 assumptions. One of these hypotheses assumes that "the law of cell-DNA synthesis is uniform over the population". In other words, on average all cells synthesise their genome by following the same dynamical path. This would be a correct approximation to extract a population-averaged behaviour, as any singularity in a particular cell would be distributed over the whole cell population. However, it would not stand if one were interested in the exact behaviour of a single cell. Indeed, the heterogeneity in DNA synthesis of individual cells can be observed by the variation of the width of cell distribution (inset in Fig 2C). While some cells start the S phase others are more advanced in their DNA replication, and therefore as the S phase progresses the width of the cell distribution increases. However, at the same time then the *I(t)* starts to decrease the width of the cell distribution decreases. This could be interpreted to mean that the overall rate of DNA synthesis decreases in cells with a higher degree of replication, but increases in cells with a lower degree of replication so that the latter catch up with the former. Nevertheless, some cells reach the G2 phase while other cells are still in S phase.

Therefore, the single cell *I(t)* and N_f*(t)* extracted by the method of Bertuzzi from our data

represents only the average envelope of these two quantities in any cell. The real single cell $I(t)$ and $N_f(t)$ are certainly different from cell to cell but in a first approximation they follow the form that we have extracted from the experimental data.

5. Characterisation of combed fibres.

We analysed 109 DNA fibres. For each combed DNA fibre BrdU tracks and total DNA were detected. Supplementary Table 1 reports the fibre number, the length of the fibre in kb, the replicated fraction and the position of consecutive detected replication forks. Figure S.4 represents the characterisation of the data. The characterised fibres have an average size of 164 kb, and the half width of the fibre size distribution is 104 kb. The smallest detected fibre is 47 kb long and the longest is 672 kb long (Fig S.4.a). The average length for BrdU tracks (Fig S.4.b) is 13.7 kb (+/- 11). The gaps (distance between adjacent BrdU tracks) size distribution is shown on Fig S.4.c. The average center-center distance between BrdU tracks (Fig S.4.d) is 36.6 kb (+/- 16) . These values are compatible with values reported by Lengronne et al (3).

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chromosome replication: genomewide replication in clb5Δ Saccharomyces cerevisiae. Genetics, 180(4), 1833– 1847.

Captions:

Fig. S1.

Facs profile of budding yeast cell population grown exponentially versus the DNA content. The open circles are experimental data points, and the solid black line is the predicted profile using equation (1) and the measured $f_{DNA}(t)$.

Fig.S2.

Fraction of replicated DNA from the microarray data versus time. In all panels the open circles are experimental data and the black solid line is the calculated fraction of replicated DNA using equation 4 and the extracted $I(t)$ profile for each data sets. **A.** Combing **B.** Raghuraman et al (8). **C**. Yabuki et al (9). **D**. McCune et al (10).

Fig.S3.

Simulated $I(t)$ and $N_F(t)$. Two situations were considered, the origin firing probability has a constant value (**A, B, C**) and the firing probability is modulated by the fork density (**D, E, F**). In both cases $I(t)$ and $N_F(t)$ were measured considering different experimental resolution Δx: Δx = 0 kb (□), Δx = 1 kb (○),Δx = 2 kb (Δ),Δx = 4 kb (∇),Δx = 6 kb (◊).

Fig.S4.

Characterisation of combed DNA fibres. **A**. Fibres size distribution. **B.** BrdU track size distribution. **C.** Distribution of distances between two adjacent BrdU tracks, gaps. **D.** Distribution of distances between the centres of two adjacent BrdU tracks.

Figures:

Fig.S1

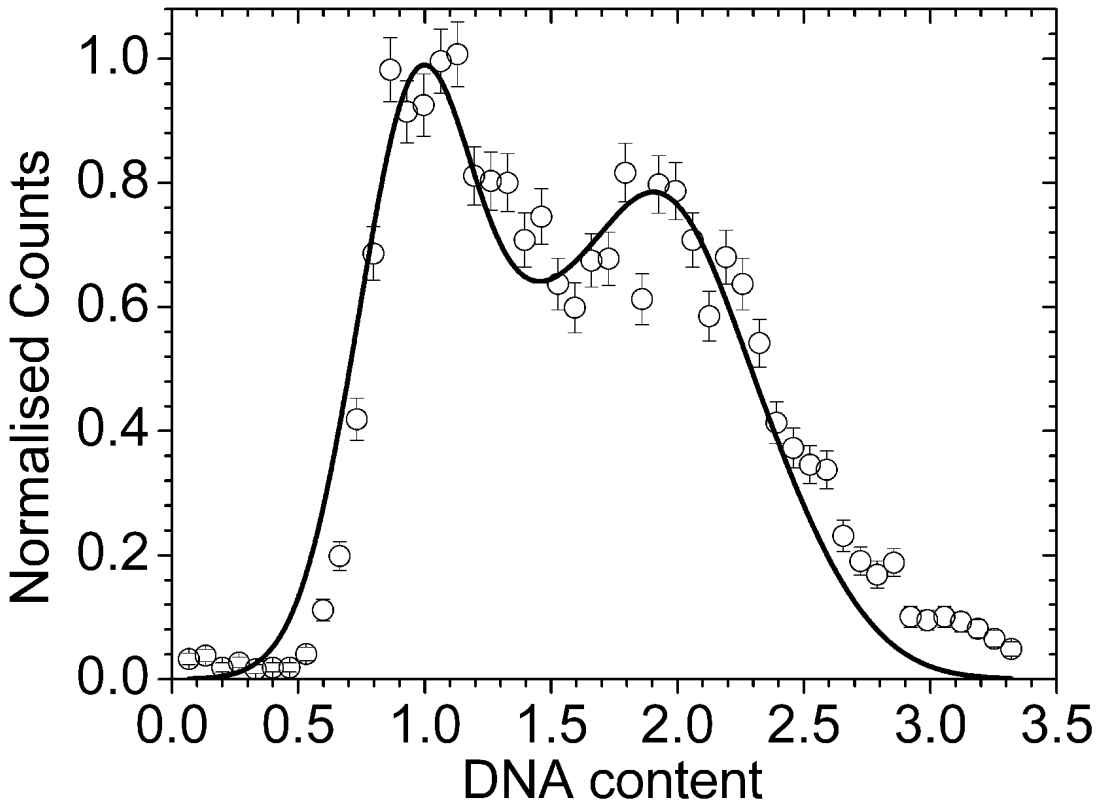

Fig.S2.

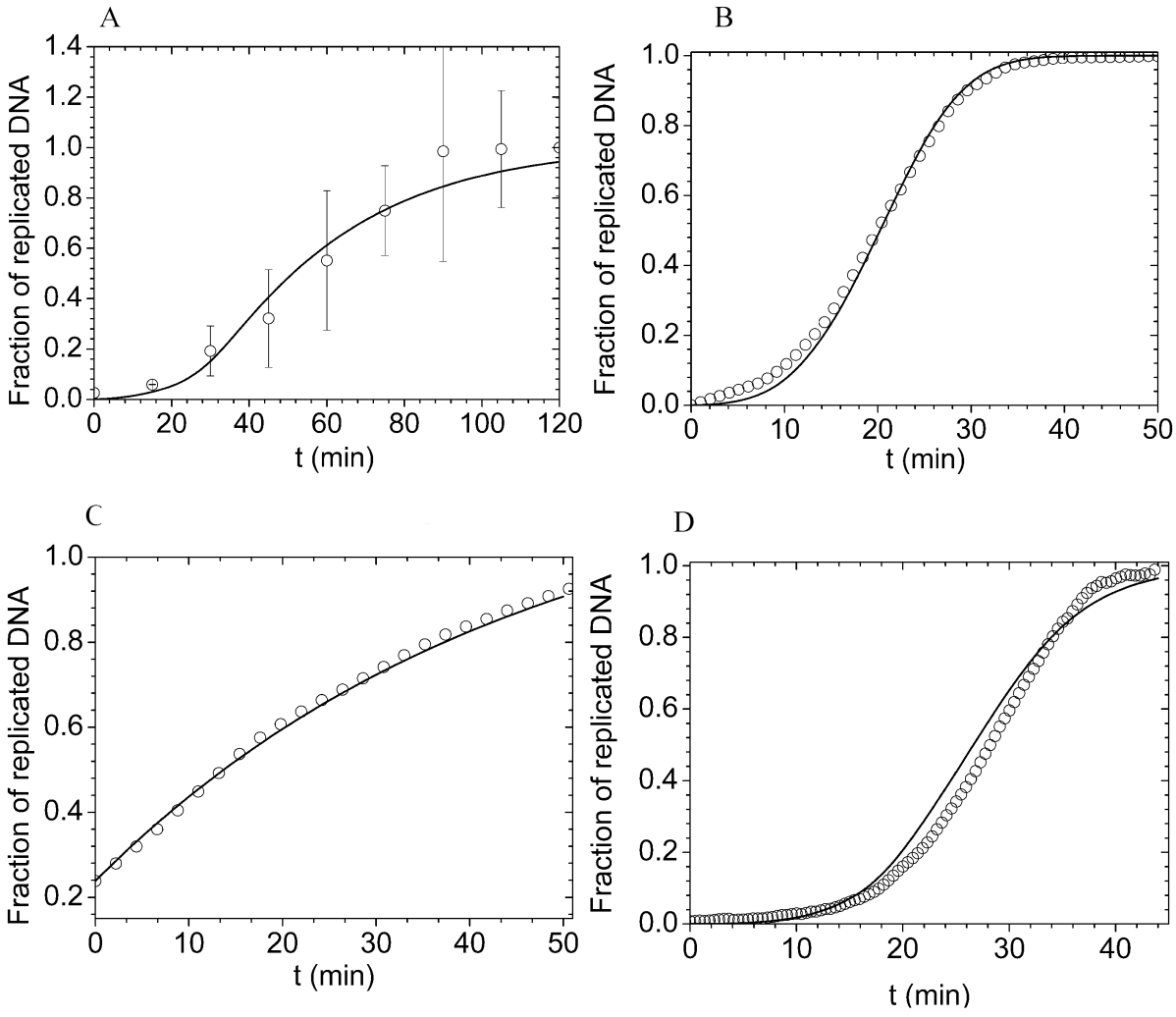

Fig.S3.

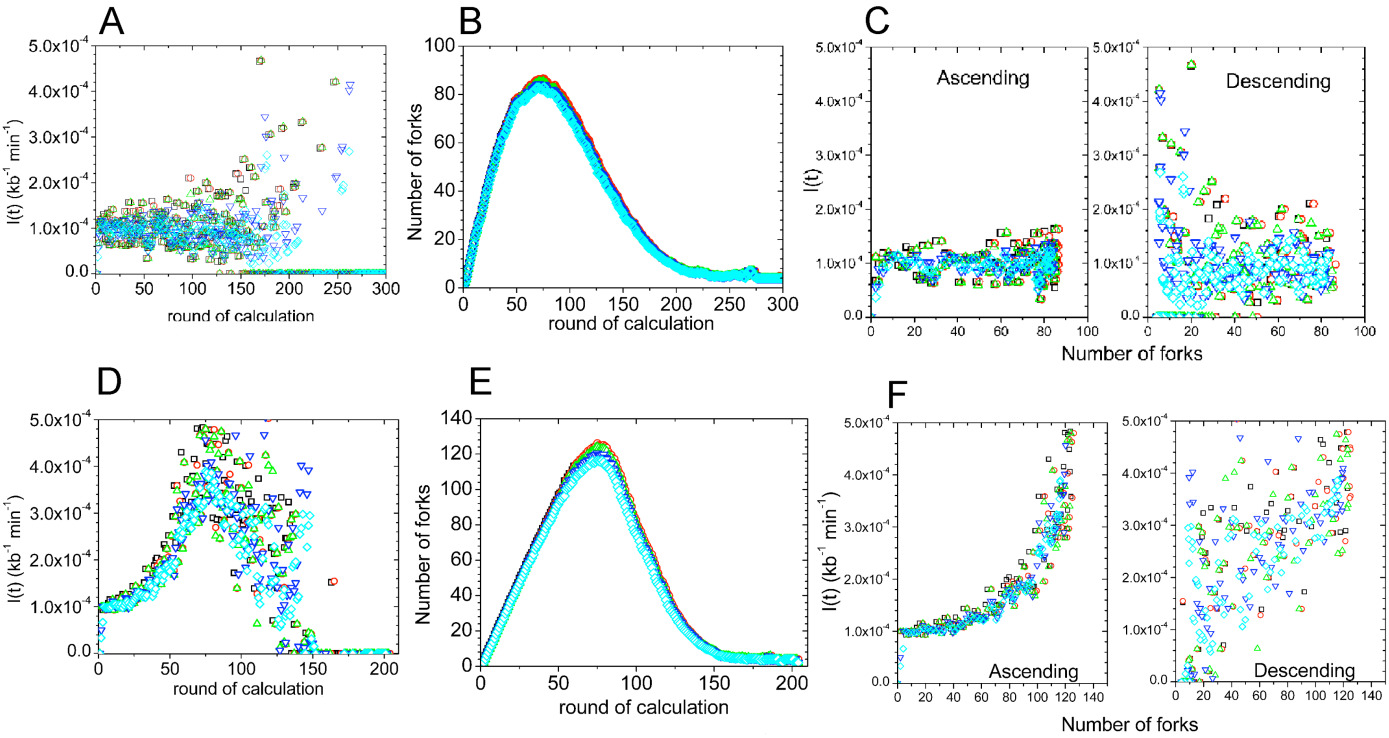
A
B
C
D
E
F
I(t) (kb^{-1} min^{-1})
round of calculation
Number of forks
I(t)
Ascending
Descending

Fig.S4.