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1 Organization of DNA replication 2 origin firing in *Xenopus* egg extracts : 3 the role of intra-S checkpoint

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14 **Abstract** During cell division, the duplication of the genome starts at multiple positions called
15 replication origins. Origin firing requires the interaction of rate-limiting factors with potential
16 origins during the S(ynthesis)-phase of the cell cycle. Origins fire as synchronous clusters is
17 proposed to be regulated by the intra-S checkpoint. By modelling either the unchallenged or the
18 checkpoint-inhibited replication pattern of single DNA molecules from *Xenopus* sperm chromatin
19 replicated in egg extracts, we demonstrate that the quantitative modelling of data require: 1) a
20 segmentation of the genome into regions of low and high probability of origin firing; 2) that regions
21 with high probability of origin firing escape intra-S checkpoint regulation; 3) that the intra-S
22 checkpoint controls the firing of replication origins in regions with low probability of firing. This
23 model implies that the intra-S checkpoint is not the main regulator of origin clustering. The minimal
24 nature of the proposed model foresees its use to analyse data from other eukaryotic organisms.

26 Introduction

27 Eukaryotic genomes are duplicated in a limited time during the S phase of each cell cycle. Replication
28 starts at multiple origins that are activated (fired) at different times in S phase to establish two
29 diverging replication forks that progress along and duplicate the DNA at fairly constant speed
30 until they meet with converging forks originated from flanking origins (*DePamphilis and Bell, 2010*;
31 *Machida et al., 2005*). The mechanisms that regulate the origin firing timing remain largely unknown
32 (*Raghuraman, 2001; Heichinger et al., 2006; Eshaghi et al., 2007; Baker et al., 2012; Audit et al.,*
33 *2013; Rhind and Gilbert, 2013*). The core motor component of the replicative helicase, the MCM2-
34 7 complex, is loaded on chromatin from late mitosis until the end of G1 phase as an inactive
35 head-to-head double hexamer (DH) to form a large excess of potential origins (*DePamphilis et al.,*
36 *2006; Ticaú et al., 2015*). During S phase, only a fraction of the MCM2-7 DHs are activated to
37 form a pair of active Cdc45-MCM2-7-GINS (CMG) helicases and establish bidirectional replisomes
38 (*DePamphilis and Bell, 2010*). MCM2-7 DHs that fail to fire are inactivated by forks emanating from
39 neighboring fired origins (*Blow et al., 2011*). Origin firing requires S-phase cyclin-dependent kinase
40 (CDK) and Dbf4-dependent kinase (DDK) activities as well as the CDK targets Sld2 and Sld3 and the

41 replisome-maturation scaffolds Dpb11 and Sld7 in *S. cerevisiae*. The six initiation factors Sld2, Sld3,
42 Dpb11, Dbf4, Sld7 and Cdc45 are expressed at concentrations significantly lower than the MCM
43 complex and core replisome components, suggesting that they may be rate-limiting for origin firing
44 (Mantiero et al., 2011; Tanaka et al., 2011). Among these six factors, Cdc45 is the only one to travel
45 with the replication fork.

46 DNA replication initiates without sequence specificity in *Xenopus* eggs (Harland and Laskey, 1980;
47 Méchali and Kearsley, 1984), egg extracts (Mahbubani et al., 1992; Hyrien and Méchali, 1992; Carli
48 et al., 2016, 2018) and early embryos (Hyrien and Méchali, 1993; Hyrien et al., 1995) (for review see
49 Hyrien et al. (2003)). To understand how a lack of preferred sequences for replication initiation
50 is compatible with a precise S-phase completion time, investigators have studied replication at
51 the single DNA molecule level using the DNA combing technique (Lucas et al., 2000; Herrick et al.,
52 2000; Blow et al., 2001; Marheineke and Hyrien, 2001, 2004). In contrast to population based
53 approaches (which average replication characteristics, this technique reveals cell-to-cell differences
54 in origin activation important for understanding how genomes are replicated during S-phase) these
55 experiments did not detect a regular spacing of initiation events but revealed that origin firing rate
56 strongly increases from early to late replication intermediates, speeding up late replication stages
57 (Lucas et al., 2000; Herrick et al., 2000). An observation that has been also confirmed for many
58 other model organisms, including human cell lines (Goldar et al., 2009).

59 Mathematical modelling based on the assumption (mean-field hypothesis) that the probability
60 of firing of each replication origin can be replaced by the averaged probability of firing calculated
61 over all degree of freedom of origin firing process (MCM2-7 DH density, genomic position, chromatin
62 compaction, nucleosome density, etc) and augmented with the assumption of independent origins
63 and a constant fork speed, allowed the extraction of a time-dependent rate of replication initia-
64 tion, $I(t)$, from the measured eye lengths, gap lengths and eye-to-eye distances on combed DNA
65 molecules (Herrick et al., 2002). The extracted $I(t)$ markedly increased during S phase. Simulations
66 incorporating this extracted $I(t)$ reproduced the mean eye length, gap length and eye-to-eye dis-
67 tance, but the experimental eye-to-eye distance distribution appeared “peakier” than the simulated
68 one (Hyrien et al., 2003; Jun et al., 2004). Modulating origin firing propensity by the probability to
69 form loops between forks and nearby potential origins resulted in a better fit to the data without
70 affecting $I(t)$ (Jun et al., 2004).

71 Importantly, experiments revealed that in *Xenopus*, like in other eukaryotes, replication eyes are
72 not homogeneously distributed over the genome but tend to cluster (Blow et al., 2001; Marheineke
73 and Hyrien, 2004). First, a weak correlation between the sizes of neighbouring eyes was observed
74 (Blow et al., 2001; Marheineke and Hyrien, 2004; Jun et al., 2004), consistent with firing time cor-
75 relations. Second, more molecules with no or multiple eyes than expected for spatially uniform
76 initiation were observed in replicating DNA (Marheineke and Hyrien, 2004). There are two potential,
77 non-exclusive mechanisms for these spatiotemporal correlations. The first one, compatible with a
78 mean-field hypothesis, is that activation of an origin stimulates nearby origins. The second one, no
79 longer consistent with a mean-field hypothesis, is that the genome is segmented into multi-origin
80 domains that replicate at different times in S phase. This second hypothesis has been explored
81 numerically in human and has been shown to be compatible with the universal bell shaped $I(t)$
82 profile (Gindin et al., 2014).

83 Interestingly, experiments in *Xenopus* egg extracts revealed that intranuclear replication foci labelled
84 early in one S phase colocalized with those labelled early in the next S phase, whereas the two
85 labels did not coincide at the level of origins or origin clusters were examined (Labit et al., 2008).
86 Given the different characteristic sizes of timing domains (1-5 Mb) and origin clusters (50-100 kb) in
87 the *Xenopus* system, it is possible that origin correlations reflect both a programmed replication
88 timing of large domains and a more local origin cross-talk within domains.

89 It is now well accepted that the intra-S phase checkpoint regulates origin firing during both
90 unperturbed and artificially perturbed S phase (Marheineke and Hyrien, 2004; Ge and Blow, 2010;
91 Guo et al., 2015; Platel et al., 2015; Forey et al., 2020). DNA replication stress, through the activation

of the S-phase checkpoint kinase Rad53, can inhibit origin firing by phosphorylating and inhibiting Sld3 and Dbf4 (Zegerman and Diffley, 2010). The metazoan functional analogue of Rad53 is Chk1. Experiments in human cells under low replication stress conditions showed that Chk1 inhibits the activation of new replication factories while allowing origin firing to continue within active factories (Ge and Blow, 2010). Experiments using *Xenopus* egg extracts suggested that the checkpoint mainly adjusts the rate of DNA synthesis by staggering the firing time of origin clusters (Marheineke and Hyrien, 2004). Recently, we showed that even during an unperturbed S phase in *Xenopus* egg extracts, Chk1 inhibits origin firing away from but not near active forks (Platel et al., 2015). We used our initial model for DNA replication in *Xenopus* egg extracts (Goldar et al., 2008) (which combined time-dependent changes in the availability of a limiting replication factor, and a fork-density dependent affinity of this factor for potential origins) to model the regulation of DNA replication by the intra-S checkpoint. To account for the regulation of DNA replication by the intra-S checkpoint, we replaced the dependency of origin firing on fork density by a Chk1-dependent global inhibition of origin firing with local attenuation close to active forks as was proposed in other contexts (Trenz et al., 2008; Dimitrova and Gilbert, 2000; Thomson et al., 2010; Ge and Blow, 2010). This model was able to simultaneously fit the $I(f)$ (the rate of origin firing expressed as a function of each molecule's replicated fraction f) of a control and a UCN-01-inhibited Chk1 replication experiment (Platel et al., 2015). However, in that work we did not push further the analysis to verify if our model was able to explain simultaneously $I(f)$ (temporal program) and the eye-to-eye distance distribution (spatial program).

In the present work, using numerical simulations, we quantitatively analyse both the temporal and spatial characteristics of genome replication as measured by DNA combing in the in vitro *Xenopus* system. The use of *Xenopus* egg extracts has been proven to study DNA replication in metazoans (Hoogenboom et al., 2017). Rooted on experimental data, we build a general and minimal model of DNA replication able to predict its temporal and spatial characteristics either during an unchallenged or a challenged S phase. By analysing the spatio-temporal pattern of DNA replication under intra-S checkpoint inhibition and comparing it to an unchallenged pattern we disentangle the complex role of the intra-S checkpoint for replication origin firing.

Results

Finding the best integrative model of unperturbed S phase

Our previous model (Platel et al., 2015) failed to simultaneously reproduce the eye-to-eye distance distribution and the $I(f)$ of the same control experiment (Figure 1 a and b). This discrepancy could be explained if initiation events have a strong tendency to cluster (Blow et al., 2001; Marheineke and Hyrien, 2004). Clustering produces an excess of small (intra-cluster) and large (inter-cluster) eye-to-eye distances compared to random initiations, but only the former could be detected on single DNA molecules due to finite length (Marheineke and Hyrien, 2004). Chk1 action has been proposed to regulate origins clusters (Ge and Blow, 2010). However, Chk1 inhibition by UCN-01 did not result in the broader eye-to-eye distribution predicted by random origin firing (Figure 1 c and d), suggesting that other mechanisms than intra-S checkpoint are involved in the origin clustering.

We therefore explored the ability of several nested models with growing complexity (designated MM1 to MM4) (Appendix 1). MM1 corresponds to a mean field hypothesis of origin firing : all potential origins have a constant firing probability P_{out} (Goldar et al., 2008; Gauthier and Bechhoefer, 2009). MM2 corresponds to MM1 with a local perturbation, whereby the proximity of forks facilitates origin firing (Jun et al., 2004; Löb et al., 2016) over a distance d downstream of an active fork where the probability of origin firing is P_{local} . In MM3 origin firing does not follow mean field hypothesis but assumes that the genome can be segmented into regions of high and low probabilities of origin firing (Gindin et al., 2014; Löb et al., 2016) as accepted for most eukaryotes (McCune et al., 2008; Yang et al., 2010; Rhind and Gilbert, 2013; Boulos et al., 2015; Das et al., 2015; Petryk et al., 2016; Siefert et al., 2017). In this scenario, the probability of origin firing of potential origins located

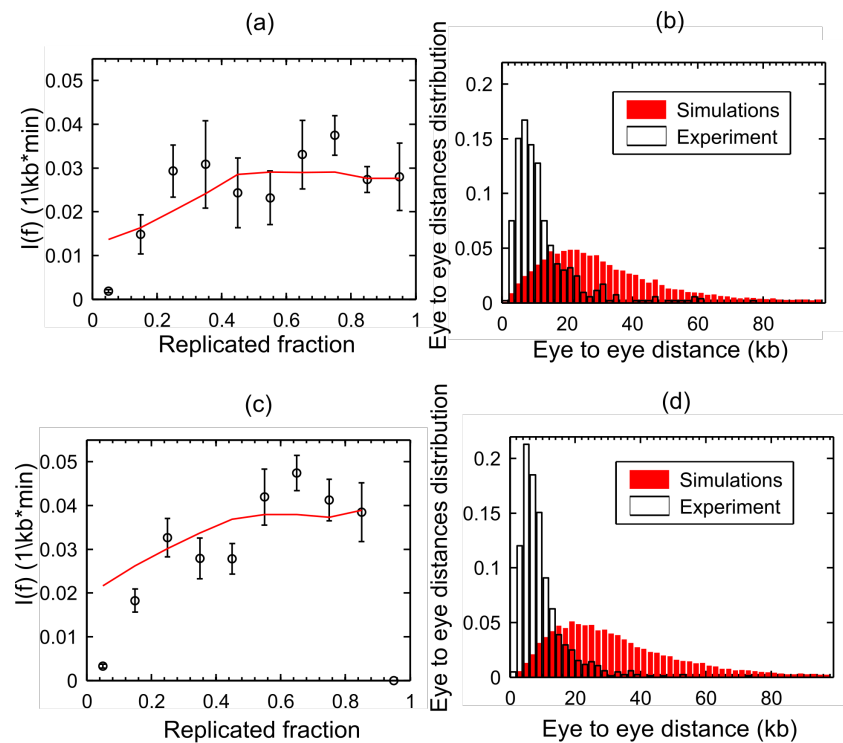


Figure 1. Chk1 does not control origin clustering. The black symbols are experimental data and the red curves are simulations. (a) and (c) Fitting of $I(f)$ data reported in *Platel et al. (2015)* for control and Chk1 inhibition experiments respectively. (b) and (d) Discrepancy between experimental and simulated distributions of eye-to-eye distances in control and Chk1 inhibition experiments, respectively.

141 within a fraction θ of the genome, P_{in} is assumed to be higher than the firing probability P_{out} of
 142 potential origins in the complementary fraction $1 - \theta$. Lastly, MM4 combines the specific features of
 143 MM2 and MM3 into a single model. Furthermore, to verify if the localized nature of potential origins
 144 (*Yang et al., 2010; Arbona et al., 2018*) can influence the spatio-temporal program of origin firing,
 145 each considered scenario was simulated assuming either a continuous or a discrete distribution of
 146 potential origins.

147 For each model, we coupled dynamic Monte Carlo numerical simulations to a genetic optimization
 148 algorithm to find the family of variables that maximized the similarity between the simulated and
 149 measured profiles of $I(f)$, replicated fraction of single molecules, global fork density, eye-to-eye
 150 distances, gap lengths and eye lengths. MM4 with localized potential origins (*Figure 2*) provided the
 151 best fit to the experimental data (Appendix 1, *Figure 8*). The increase in concordance between MM4
 152 and the data occurs at the expense of increasing the number of parameters, which is justifiable on
 153 statistical grounds (Appendix 1, *Table 2*).

154 **Verifying the predictive ability of MM4 model**

155 The real DNA replication process is far more complex than any of the above models. To explore how
 156 accurately MM4 can map a more complex process, we built, based on replication process in other
 157 eukaryotes (*McCune et al., 2008; Yang et al., 2010; Rhind and Gilbert, 2013; Boulos et al., 2015;*
 158 *Das et al., 2015; Petryk et al., 2016; Siefert et al., 2017*) and our previous model (*Platel et al., 2015*),
 159 a more elaborate model (MM5, Appendix 2) to generate *in silico* data with 8%, 19% and 53% global
 160 replicated fractions. MM5 assumes that the replication pattern of the genome is reproduced by the
 161 coexistence between regions with low probability of origin firing and localised domains with higher
 162 probability of origin firing, furthermore MM5 includes explicitly the effect of intra-S checkpoint
 163 through supplementary probabilities of origin firing inhibition. However, as during combing experi-
 164 ment the genome is broken randomly into smaller molecules the positional information of each

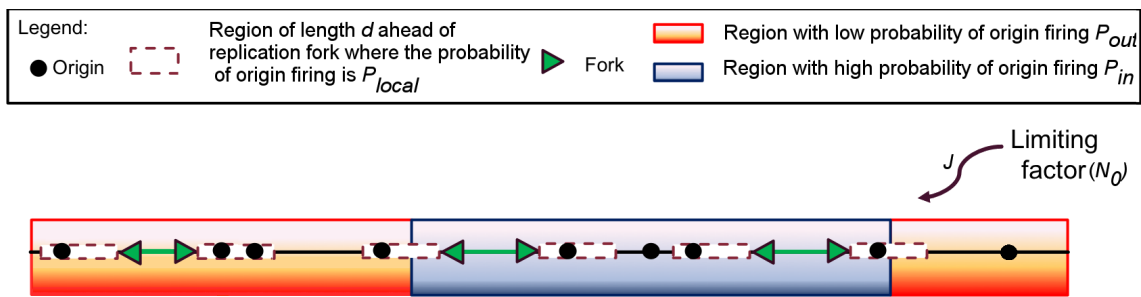


Figure 2. Schematic representation of MM4. Potential replication origins located in a fraction θ of the genome (not necessary contiguous) have a probability of firing P_{in} higher than probability of firing P_{out} of potential origins located in the complementary genome fraction $1 - \theta$. The firing of a potential origins requires its encounter with limiting factors which number $N(t) = N_0 + Jt$ increases as S phase progresses. Potential origins fire with a probability P_{local} over a distance d ahead of a replication fork.

165 combed single molecule is lost and therefore only genome averaged information can be extracted
 166 from a traditional combing experiment. We calculated the expected genome averaged values for
 167 each parameter of MM5 (Appendix 2, "Reduction of MM5 to MM4"). Each sample was then fitted
 168 with MM4 (Appendix 2 **Figure 1**, **Figure 2** and **Figure 3**) and we compared the extracted parameters
 169 with their expected values after reduction of MM5 to MM4 (**Figure 3**; Appendix 2, **Table 3**).

For each sample, the mean values of the inferred parameters were statistically similar to the input

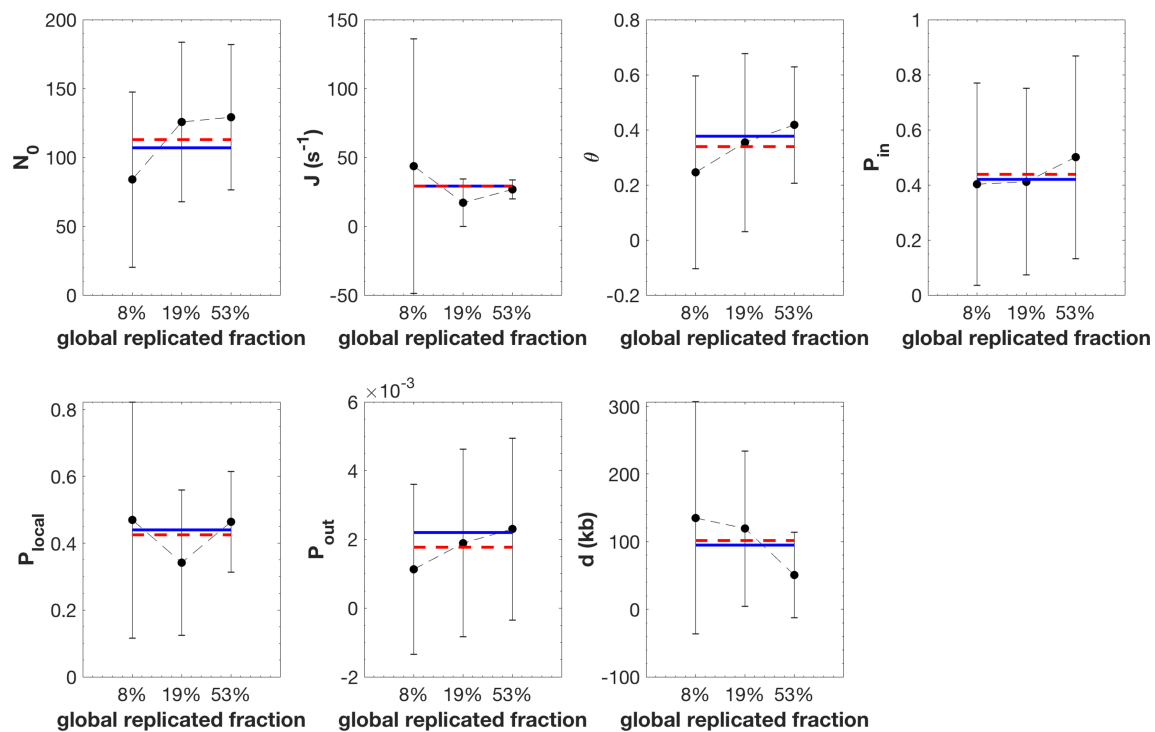


Figure 3. The fitting strategy infers accurately the expected values for the reduced MM5 free parameters. The black circles correspond to the averaged value of the parameter over 100 independent fits and the error bars are the standard-deviations. The solid blue line is the expected value of the parameter as obtained in Appendix 2, **Table 3**. The red dashed line is the mean value of the parameter obtained by averaging the parameter inferred values over the 3 samples.

170
 171 ones (Appendix 2, **Table 3**) and none of the pairwise differences between the predicted parameters
 172 values for the 3 considered samples were statistically significant. This demonstrates that our
 173 fitting and comparison strategies do not introduce artificial differences between parameters if
 174 their values do not change between different samples (Appendix 2 **Figure 4**). In conclusion, any

175 variation in parameter value detected by MM4 when analysing samples at different time points
 176 independently can be considered as statistically significant. Therefore, MM4 can adequately model
 177 more complex DNA replication dynamics than itself using a reduced number of parameters.

178 Retrieving the dynamics of an unchallenged S phase using the MM4 model

179 MM4 faithfully reproduced the temporal and spatial program of DNA replication from unperturbed S
 180 phase samples with global replicated fractions of 8%, 19% and 53% (Appendix 1, **Figure 8**; Appendix
 181 3, **Figure 1** and **Figure 2**). The fitted values of parameters changed as S phase progressed (**Figure 4**).
 However, only changes in J , θ , P_{out} and d were statistically significant (Appendix 3 **Figure 3**). In

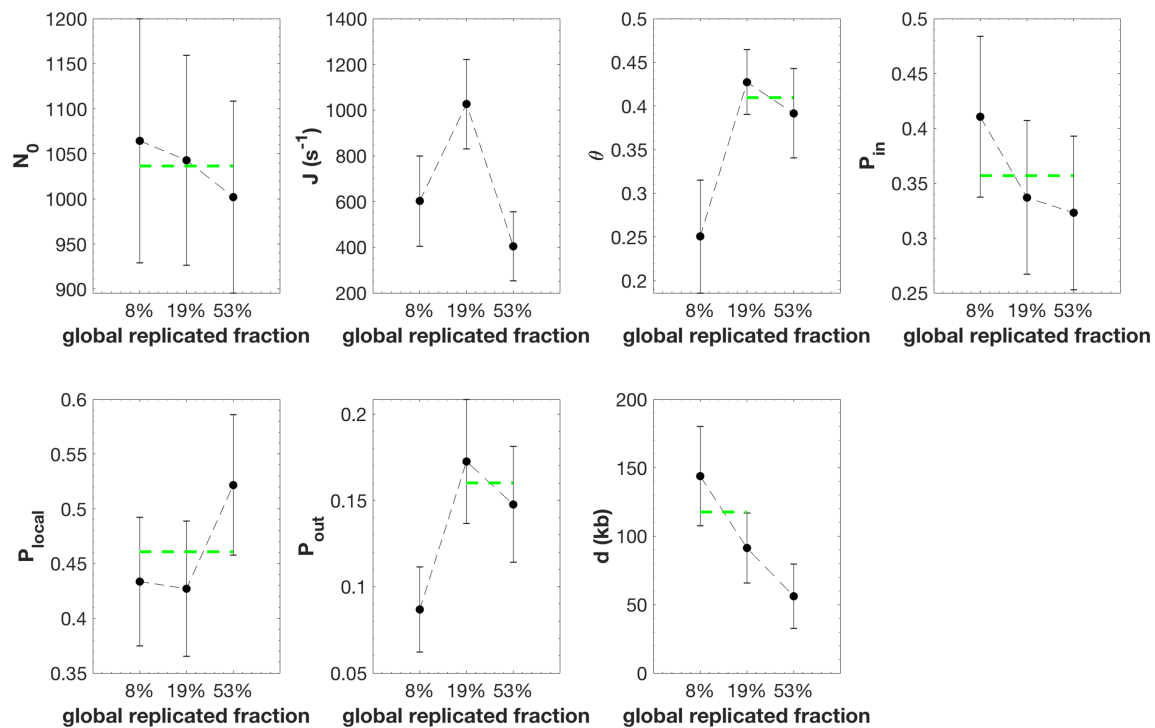


Figure 4. Inferred model parameters by fitting unchallenged S phase data as global replicated fraction increases. The black circles are the averaged value of the parameter over 100 independent fitting processes and the error bars are standard-deviations. The green dashed line is the mean value among consecutive parameters which differences are not statistically significant (Appendix 3 **Figure 3**).

182 particular we found that J increased from 8% to 19% replication and then drop back at 53%
 183 replication. θ and P_{out} increased only from 8% to 19% replication but not later, while d stayed
 184 constant between 8% and 19% replication and decreased at 53% replication.

185 These observations suggest that during an unchallenged S phase both the fraction (θ) of the genome
 186 with high probability of origin firing and the background probability (P_{out}) of origin firing outside that
 187 fraction increase as S phase progresses. Interestingly, P_{local} is higher than P_{in} and P_{out} , suggesting
 188 that firing of an potential origin significantly favours the firing of nearby potential origins over a
 189 distance d , compatible with a chromatin looping process (Löb *et al.*, 2016). This fork-related firing
 190 process is consistent with the observation that nearby origins tend to fire at similar times, which
 191 has been proposed to result from a different regulation of nearby and distant origins by Chk1 (Ge
 192 and Blow, 2010; Platel *et al.*, 2015).
 193

194 Modeling DNA replication under Chk1 inhibition

195 To decipher the regulation of origin firing by Chk1, we examined if the MM4 model could also
 196 reproduce the replication program observed when the intra-S phase checkpoint was inhibited
 197 by the specific Chk1 inhibitor UCN-01. We analyzed combed fibres from a replicated sample in

198 the presence of UCN-01 (replicated fraction 22%) that had spent the same interval of time in S
 199 phase as the control sample (global replicated fraction of 8%). The MM4 model reproduced the
 experimental observations very well (Appendix 3, **Figure 4**, $GoF_{global} = 0.85$). The three

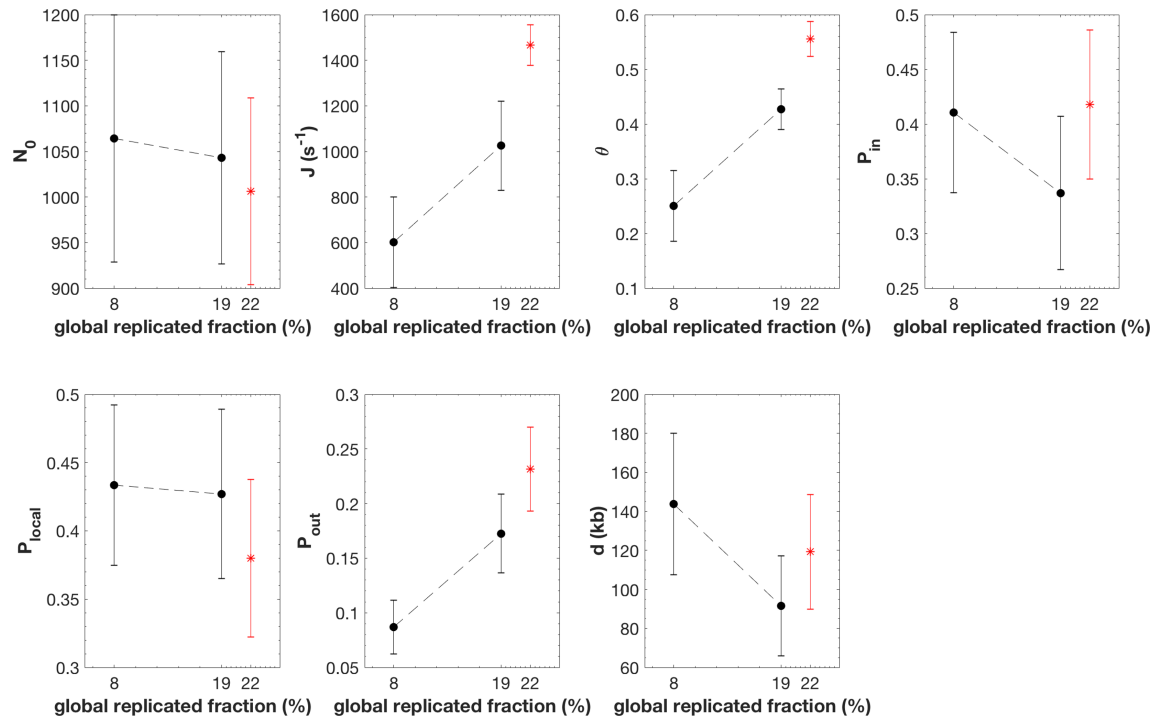


Figure 5. J , θ , and the P_{out} are the only parameters that change when comparing unchallenged and Chk1 inhibited S phase The black circle is the averaged value of the parameter over 100 independent fitting processes of unchallenged S phase and the error bars are standard-deviations. The red star is the averaged value of the parameter over 100 independent fitting processes of Chk1 inhibited sample and the error bars represent the standard-deviations.

200
 201 parameters J , θ , and the P_{out} were significantly higher in the UCN-01 treated sample than in the
 202 control samples with either the same harvesting time or a similar replicated fraction (22% and 19%,
 203 respectively) (**Figure 5** and Appendix 3 **Figure 5**). The other parameters were unchanged compared
 204 to both control samples. These results suggest that upon Chk1 inhibition (i) a fraction θ of the
 205 genome, where initiation probability is high, increases during S phase; (ii) the probability of origin
 206 firing is insensitive to Chk1 within this fraction (P_{in} is unaltered) but is increased in the rest of the
 207 genome (P_{out} is increased) ; (iii) the import/activation rate of the limiting factor, J , is increased,
 208 while the starting number of factors, N_0 , is unaffected. As was expected, MM4 detected that Chk1
 209 inhibition by UCN-01 increased origin firing (*Platel et al., 2015; Syljuasen et al., 2005; Guo et al.,*
 210 *2015; Michelena et al., 2019; Pommier and Kohn, 2003; Deneke et al., 2016*).
 211 In conclusion , the level of active Chk1 appears to regulate the kinetics of S phase progression (i) by
 212 limiting the genome fraction that escapes its inhibitory action, (ii) by down regulating the probability
 213 of origin firing outside this fraction (*Syljuasen et al., 2005; Maya-Mendoza et al., 2007; Guo et al.,*
 214 *2015; Michelena et al., 2019*), and (iii) by controlling the import/activation rate of limiting firing
 215 factors (*Guo et al., 2015*). However, no significant differences in the strength of origin regulation
 216 by nearby forks (P_{local}) was observed after Chk1 inhibition, suggesting that this local action is not
 217 mediated by Chk1 (*Trenz et al., 2008; Ge and Blow, 2010*).

218 Discussion

219 We explored several biologically plausible scenarios to understand the spatio-temporal organization
 220 of replication origin firing in *Xenopus* egg extracts. We used a quantitative approach to objectively

221 discriminate which model best reproduced the genomic distributions of replication tracks as
222 analyzed by DNA combing at different stages of S phase. We found that model MM4 with discrete
223 potential origins best reproduced the experimental data with a minimal number of adjustable
224 parameters. This model combines five assumptions (*Herrick et al., 2002; Goldar et al., 2008;*
225 *Gauthier and Bechhoefer, 2009; Blow and Ge, 2009; Sekedat et al., 2010; Yang et al., 2010; Platel*
226 *et al., 2015; Löb et al., 2016; Gindin et al., 2014; Arbona et al., 2018*): 1) origin firing is stochastic,
227 2) the availability of a rate-limiting firing factor captures the essential dynamics of the complex
228 network of molecular interactions required for origin firing, 3) the speed of replication forks is
229 constant 4) origins fire in a domino-like fashion in the proximity of active forks (*Guilbaud et al.,*
230 *2011; Löb et al., 2016*); 5) the probability of origin firing is heterogeneous along the genome (*Yang*
231 *et al., 2010; Gindin et al., 2014*).

232 We used MM4 to model DNA combing data from *Xenopus* egg extracts in presence or absence of
233 intra-S checkpoint inhibition. In both conditions, this model was able to match the experimental
234 data in a satisfactory manner. Furthermore, the inferred parameters values indicated that the
235 global probability of origin firing and the rate of activation/import of the limiting firing factor (J)
236 were increased after Chk1 inhibition by UCN-01 (*Pommier and Kohn, 2003; Seiler et al., 2007; Guo*
237 *et al., 2015*). Importantly, this model assumes a heterogeneous probability of origin firing and
238 suggests that Chk1 exerts a global origin inhibitory action during unperturbed S phase (*Platel et al.,*
239 *2015*). On the other hand, the constancy of the initial number of limiting factors N_0 in the presence
240 or absence of UCN-01 suggests that Chk1 does not actively control origins before S phase actually
241 starts (*Lupardus et al., 2002; Stokes et al., 2002; Forey et al., 2020*). These observations indicate
242 that MM4 can deliver a reliable, minimally complex picture of origin firing regulation in *Xenopus* egg
243 extracts.

244 **The global inhibition of origin firing by Chk1**

245 We previously showed that Chk1 is active and limits the firing of some potential origins in an
246 unperturbed S phase (*Platel et al., 2015*). Therefore, the earliest origins must be immune to Chk1
247 inhibition while later potential origins are strongly inhibited. The comparison between the modelling
248 of Chk1 inhibition and of unperturbed S phase data suggests that i) the probability of origin firing
249 is reduced by active Chk1 in a fraction $1 - \theta$ of the genome, ii) in this Chk1-sensitive fraction the
250 probability of origin firing increases as S phase progresses and iii) the probability of origin firing
251 is unaffected by Chk1 inhibition within the Chk1-immune, θ fraction of the genome. Therefore,
252 this model supports the idea that at the start of S phase, some origins fire unimpeded by Chk1,
253 whereas others remain silent. The latter only becomes progressively relieved from Chk1 inhibition
254 as S phase progresses. Indeed, recent works in cultured mammalian cells (*Moiseeva et al., 2019*),
255 *Drosophila* (*Deneke et al., 2016*) and *Xenopus* (*Krasinska et al., 2008*) showed that in unperturbed S
256 phase the global origin firing inhibitory effect (by Chk1 and Rif1) is reduced as S phase progresses.
257 Interestingly, a recent study in unperturbed yeast cells suggests that dNTPs are limiting at the
258 entry into S phase, so that, similar to *Xenopus* (*Zou, 2007*), the firing of the earliest origins creates a
259 replication stress that activates the Rad53 checkpoint which prevents further origin firing. Rad53
260 activation also stimulates dNTP synthesis, which in turn down regulates the checkpoint and allows
261 later origin firing (*Forey et al., 2020*). However, it remains uncertain if this feed-back loop does also
262 exist in *Xenopus* egg extracts which contain an abundant pool of dNTPs.

263 A key mechanism of our model is the enhancement of origin firing close to active forks. The
264 necessity to introduce this mechanism supports the domino-like view of DNA replication progression
265 (*Guilbaud et al., 2011; Löb et al., 2016*). It was previously shown in *Xenopus* egg extracts that the
266 probability of origin firing could depend on the distance between left and right approaching forks
267 (*Jun et al., 2004*). While this could in principle reflect an origin firing exclusion zone ahead of
268 forks (*Lucas et al., 2000; Löb et al., 2016*), our model did not allow for a negative P_{local} . Other
269 proposed mechanisms for origin clustering include the relief of Chk1 inhibition ahead of active
270 forks by checkpoint recovery kinase polo like kinase 1 (Plk1) (*Trenz et al., 2008; Platel et al., 2015*).

271 However, we find that the range, d , and the strength, P_{local} , of origin stimulation by nearby forks,
272 were both insensitive to checkpoint inhibition (**Figure 5** a and b). Other potential mechanisms such
273 as propagation of a supercoiling wave ahead of forks may better explain this insensitivity to Chk1
274 inhibition (**Achar et al., 2020**).

275 **Heterogeneous probability of origin firing**

276 In this model, the origin firing process in *Xenopus* egg extracts is not fiably described by a mean-
277 field approximation. In other words, the probability of origin firing is heterogeneous along the
278 genome. Based on this hypothesis, one important outcome of our study is that the genome can
279 be segmented into domains where origin firing probability is either high and immune to Chk1
280 inhibition or subjected to a tight Chk1 control that attenuates as S phase progresses. This picture
281 challenges the common view that the embryonic *Xenopus in vitro* system would lack the temporal
282 regulation by the intra-S checkpoint at the level of large chromatin domains in contrast to findings in
283 somatic vertebrate cells where Chk1 controls cluster or replication foci activation (**Maya-Mendoza**
284 **et al., 2007**). However, observations of replicating nuclei in *Xenopus* system have shown that
285 early replication foci are conserved in successive replication cycles, supporting the heterogeneous
286 domain hypothesis (**Labit et al., 2008**). Furthermore, we found that the fraction of the genome
287 covered by these domains increases and that the inhibitory action of Chk1 decreases over time
288 during an unperturbed S phase (**Figure 4** and **Figure 5**), consistent with the idea that as S phase
289 progresses more regions of the genome evade the checkpoint inhibition of origins. By comparing
290 samples that have spent the same time interval in S phase or that have reached the same replicated
291 fraction in the absence and presence of UCN-01 (**Figure 5**), we noticed that the probability of origin
292 firing in the Chk1-immune domains (P_{in}) did not change upon Chk1 inhibition. This further suggests
293 that these domains escape actually the regulation of origin firing by Chk1 that rules the rest of the
294 genome.

295 All together the results of our modelling approach and the existing literature suggest that in the
296 *Xenopus* system the position of early replicating, Chk1-immune domains is conserved in individual
297 nucleus. However, there is no experimental or numerical evidence that the positions of these
298 domains are conserved in a population of nuclei. Assuming that the position of these domains
299 changes randomly from one nucleus to another would result in a flat mean replication timing
300 pattern and involves that each nucleus has its specific replication regulation process. While we
301 cannot reject such a hypothesis objectively, the recent report of a structured replication timing
302 program in zebrafish early embryos (**Siefert et al., 2017**) encourage us to assume that in *Xenopus*
303 early embryos the position of early replication domains are conserved from one nucleus to an
304 other. Thus, we propose that the mean replication timing pattern of *Xenopus* sperm nuclei in egg
305 extracts is not flat but is structured similarly to other eukaryotic systems (**Baker et al., 2012; Rhind**
306 **and Gilbert, 2013; Boulos et al., 2015**).

307 The generality of assumptions and conclusions of our model suggest that it can be used to analyze
308 the dynamics of S phase and its regulation by the intra-S phase checkpoint in other organisms.

309 **Methods and Materials**

310 Monte Carlo simulation of DNA replication process.

311 A dynamical Monte Carlo method was used to simulate the DNA replication process as before
312 (**Goldar et al., 2008**). We simulate the replicating genome as a one-dimensional lattice of $L = 10^6$
313 blocks of value 1 for replicated and 0 for unreplicated state, respectively. To match the spatial
314 resolution of DNA combing experiments each block represents 1kb. After one round of calculation
315 an existing replication track grows in a symmetric manner by 2 blocks. Considering that the fork
316 speed $v = 0.5 \text{ kb.min}^{-1}$ is constant, one round of calculation corresponds to 2 minutes. In the
317 continuous case we assume that the potential replication origins are continuously distributed on
318 the genome with an average density of one potential origin per 1kb (1 block); in the discrete case we

319 assume that potential origins are randomly distributed along the genome with an average density of
320 one potential origin per 2.3 kb (*Edwards et al., 2002*). In both cases origins fire stochastically. Origin
321 firing requires an encounter with a trans-acting factor which number $N(t)$ increases as S phase
322 progresses with a rate J , $N(t) = N_0 + Jt$. If an encounter produces an origin firing, the trans-acting
323 factor is sequestered by replication forks and hence the number of available trans-acting factor is
324 $N_f(t) = N(t) - N_b(t)$, where $N_b(t)$ is the number of bound factors. To ensure that origins do not
325 re-fire during one cycle and are inactivated upon passive replication, only "0" blocks are able to fire.
326 At each round of calculus, each block is randomly assigned 2 independent values between 0 and
327 1. The first one is compared to θ to decide whether the block belongs to the θ or $1 - \theta$ fraction of
328 the genome. The second one to P_{in} or P_{out} , respectively, to decide whether the block may fire. In
329 total, M "0" blocks ($M \leq L$) with value strictly smaller than their reference probability may fire. If
330 $M \leq N_f(t)$ all M blocks may fire, otherwise $N_f(t)$ blocks may fire. Furthermore in MM2 and MM4,
331 we consider that the probability of origin firing P_{local} may be increased downstream of a replication
332 fork over a distance d_{fork} . The trans-acting factors sequestered by forks are released and are made
333 available for new initiation events when forks meet.

334 Measuring: the replicated fraction $f(t)$, the rate of origin firing $I(t)$, fork density $N_{fork}(t)$,
335 eye-to-eye, eye and gap length distributions.

336 The genome is represented as an one-dimensional lattice of 10^6 elements $x_i \in \{0, 1\}$. At each round
337 of calculation the replicated fraction is calculated as $f(t) = \langle x \rangle_t$, corresponding to the average value
338 of x_i over the genome.

339 The rate of origin firing per length of unreplicated genome per time unit (3 min) is calculated at each
340 round of calculation, by counting the number of newly created "1" blocks, N_1 and $I(t) = \frac{N_1}{(1-f(t))L\Delta t}$
341 where $\Delta t = 3 \text{ min}$ and $L = 10^6$. The density of replication forks is calculated at each round of
342 calculation by counting the number of "01" tracks, N_{left} , and "10" tracks, N_{right} and $N_{forks}(t) =$
343 $\frac{N_{right} + N_{left}}{L}$. The distributions of eye-to-eye distances, eye lengths and unreplicated gap sizes are then
344 computed from the distribution of "0" and "1" tracks after reshaping the data (see below).

345 Comparing experimental and numerical data.

346 The simulation results were compared to the DNA combing data from *Platel et al. (Platel et al.,*
347 **2015)**. The fluorescence intensities for total DNA and replicated tracks of each fiber were measured
348 and binarized on a Matlab® platform by using a thresholding algorithm. The threshold value
349 was chosen to minimize the difference between the replicated fraction measured by α 32P-dATP
350 incorporation and by DNA combing. Replicated tracks larger than 1kb were scored as eyes. Gaps
351 were considered significant if $> 1\text{kb}$, otherwise the two adjacent eyes were merged. The eyes
352 whose lengths span from 1 to 3 kb were considered as new origin firing events. The time interval
353 in which these new detectable events can occur was calculated as $\Delta t = 3\text{min}$ assuming a constant
354 replication fork velocity of $v \approx 0.5 \text{ kb.min}^{-1}$. This data reshaping protocol was also applied to
355 simulated DNA molecules, in order to match the spatial and temporal resolutions between the
356 experimental and simulated data. The global replicated fraction of each sample was computed as
357 the sum of all eye lengths divided by the sum of all molecule lengths. To minimize finite molecule
358 length effects in comparisons between data and simulations, the experimental molecule length
359 distribution was normalised and considered as probability density of molecule length in the sample
360 and used to weight the random shredding of the simulated genome at each time (**Figure 6**). The
361 global replication fraction of simulated cut molecules was calculated. Only molecules from the
362 simulation time that had the same global replication fraction as the experimental sample were
363 further considered.

364
365 Molecules were sorted by replicated fraction $f(t)$. The rate of origin firing and fork density
366 were calculated for each molecule as a function of $f(t)$ ($I(f)$ and $N_{fork}(f)$, respectively) for both
367 simulated and experimental data. The experimental $I(f)$, $N_{fork}(f)$, eye-to-eye distances, eye and

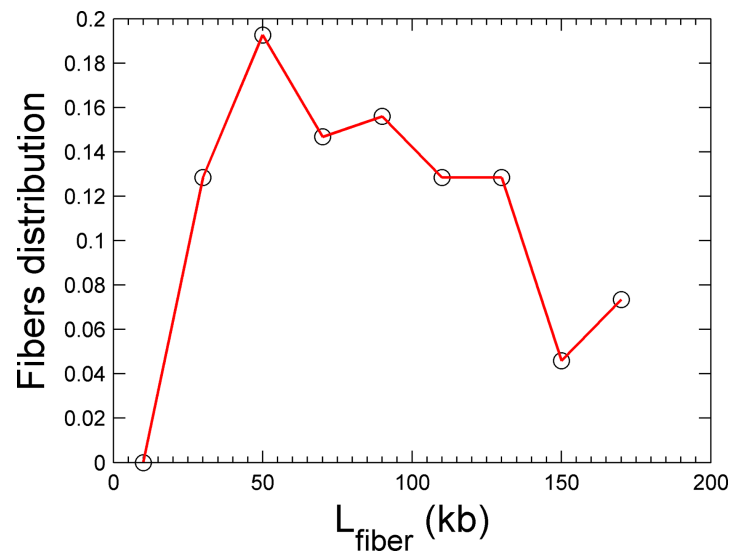


Figure 6. Molecular length distribution (global replicated fraction of 8%). The black open circles are the experimentally measured and the red curve is the simulated cut molecular length distributions, respectively .

368 gap length distributions were computed as the averaged value of three independent experiments.

369 Modeling experimental data: parameters optimization.

370 To estimate the parameters of the model, we fitted the six experimental observables ($I(f)$, $N_{fork}(f)$,
 371 replicated fiber, eye-to-eye distances, eye and gap length distribution) using a genetic optimization
 372 algorithm (Matlab®). The fitness function was defined as the sum of the square of the differences
 373 between experimental and simulated data curves divided by the squared mean of the experimental
 374 data curve. The genetic optimization algorithm was set over three subpopulations of 20 individuals
 375 with a migration fraction of 0.1 and a migration interval of 5 steps. Each individual defined a set
 376 of variables for the simulation and the variables were chosen within the range reported in **Table 1**
 377 for the model that best fit the data. At each generation, 3 elite children were selected for the next
 378 generation. The rest of the population corresponds to a mixture between 60% of children obtained
 379 after a scattered crossover between two individuals selected by roulette wheel selection and 40%
 380 of children obtained by uniform mutation with a probability of 0.2, leading to a variability of 8%.
 381 The genetic algorithm was stopped after 50 generations corresponding to the convergence of the
 382 optimization method. As the size of variable space is unknown, we considered a large domain
 383 of validity for the variables. This has as an effect to reduce the probability that the optimization
 384 process reaches a unique global minimum. For this reason we repeat the genetic optimization
 385 method 100 times independently over each data set and consider for each optimization round only
 the best elite individual.

Table 1. Lower and upper bounds of adjustable variables.

Variable	Lower bound	Upper bound	Significance
N_0	1	2000	Initial number of limiting-factor
J (s^{-1})	0	4000	Rate at which the number of limiting-factor increases
P_{out}	0	1	Probability of origin firing in the $1 - \theta$ fraction
P_{in}	0	1	Probability of origin firing in the θ fraction
P_{local}	0	1	Probability of origin firing ahead of an active replication fork over a distance d
θ	0	1	Fraction of genome where the probability of origin firing is P_{in}
d (kb)	0	1000	Distance over which a fork acts on the probability of origin firing

386

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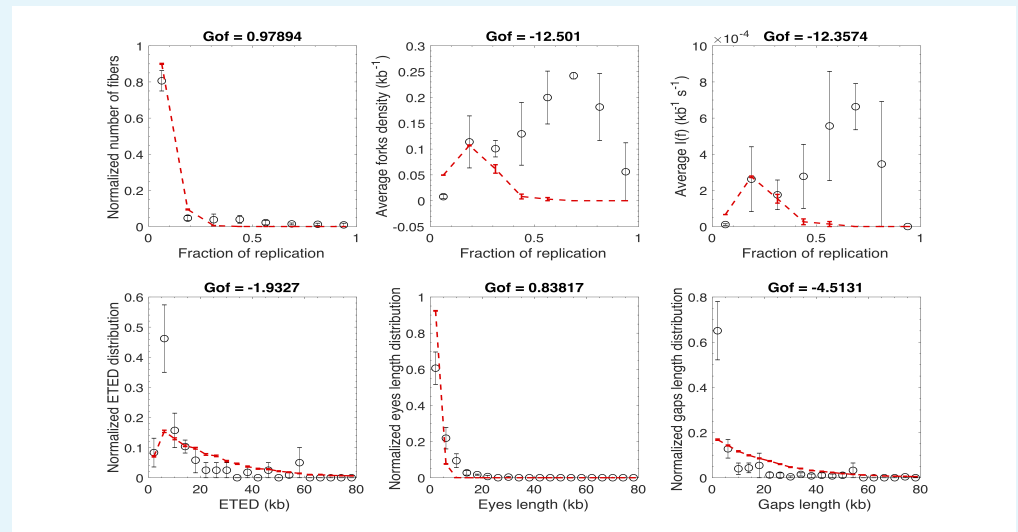
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561 Appendix 1

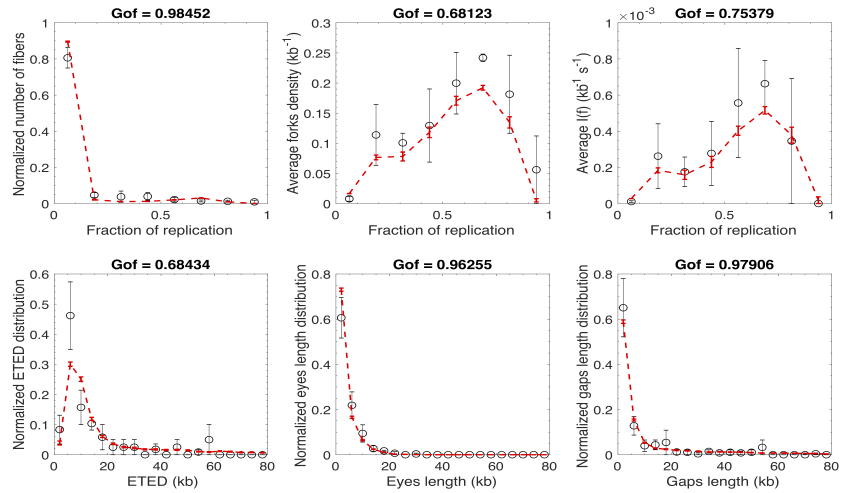
562 Different models

563 To model experimental observations a series of nested models were compared with ex-
564 perimental data. Below are the fits of each model to experimental sample with 8% global
565 replicated fraction. To assess the goodness of the fit (GoF) we considered the normalised
566 mean square error between the simulated profile and the fitted entity as the indicator of
567 likelihood ($GoF = 1 - \frac{\|y_{fit} - y_{exp}\|^2}{\|y_{exp} - \langle y_{exp} \rangle\|^2}$). GoF costs vary between $-\infty$ (bad fit) to 1 (perfect fit). If
568 $GoF = 0$, y_{fit} is no better than a straight line at matching experimental data. The global
569 cost is calculated as $GoF_{global} = \frac{1}{6} \sum_1^6 GoF_i$ where i represents one fitted entity. All models
570 reproduce with the same accuracy the distribution of replicated fibres, gaps lengths and
571 eyes lengths distributions. The major contributions to score values come from residuals
572 of average fork density, average $I(f)$ and eye-to-eye distances distribution fits. From the
573 value of GoF_{global} (Appendix1, **Table 1**), the model that best described the whole data set
574 is the MM4 with localized distribution of potential origins: its GoF_{global} value is closest to
575 one. However, MM4 also has the highest number of fitting variables (7) compared to other
576 models (MM1 has 3 fitting variables, MM2 and MM3 have 5 fitting variables), and facilitating
577 fit to the data.



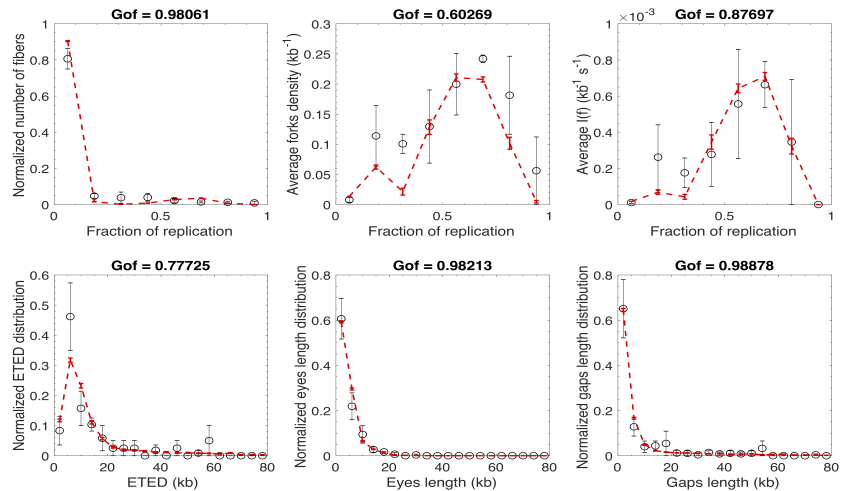
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Appendix 1 Figure 1. Modeling experimental data with MM1 model in the case where the potential origins are continuously distributed along the genome. Open circles are experimental data and the red dashed line is the fit.



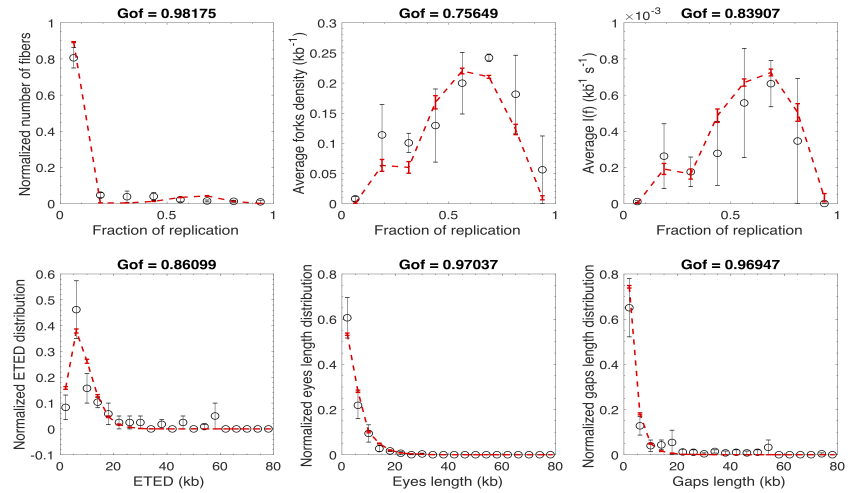
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Appendix 1 Figure 2. Modeling experimental data with MM2 model in the case where the potential origins are continuously distributed along the genome. Open circles are experimental data and the red dashed line is the fit.



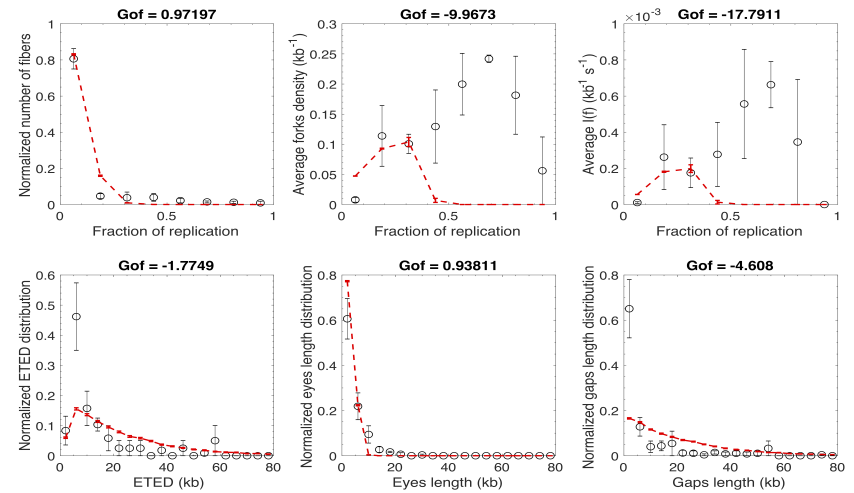
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Appendix 1 Figure 3. Modeling experimental data with MM3 model in the case where the potential origins are continuously distributed along the genome. Open circles are experimental data and the red dashed line is the fit.



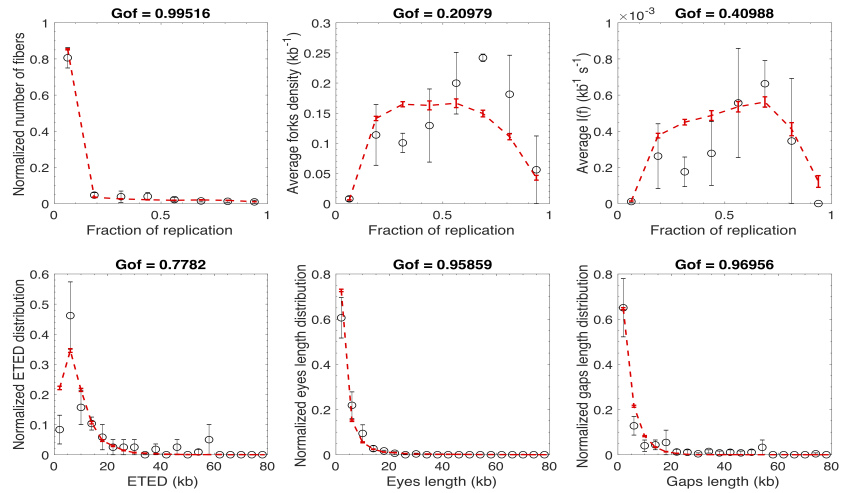
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Appendix 1 Figure 4. Modeling experimental data with MM4 model in the case where the potential origins are continuously distributed along the genome. Open circles are experimental data and the red dashed line is the fit.



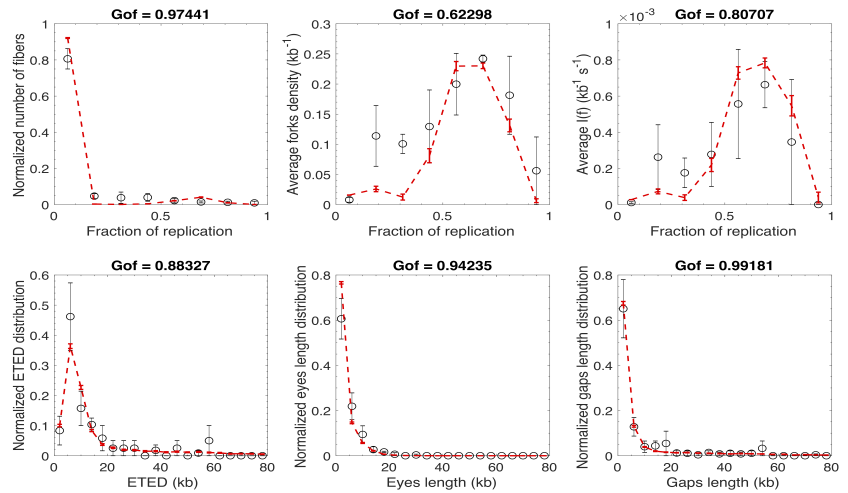
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Appendix 1 Figure 5. Modeling experimental data with MM1 model in the case where the potential origins form a discrete set along the genome. Open circles are experimental data and the red dashed line is the fit.



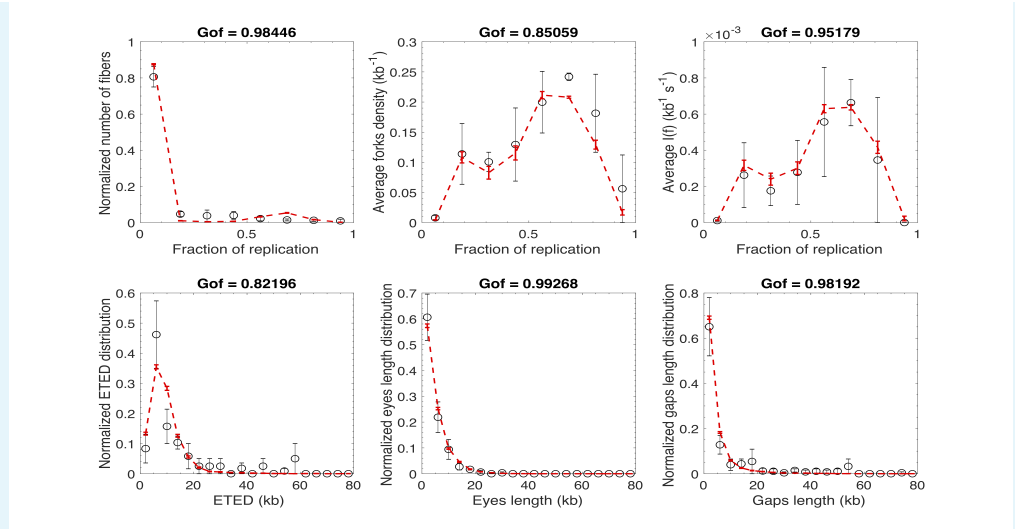
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Appendix 1 Figure 6. Modeling experimental data with MM2 model in the case where the potential origins form a discrete set along the genome. Open circles are experimental data and the red dashed line is the fit.



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Appendix 1 Figure 7. Modeling experimental data with MM3 model in the case where the potential origins form a discrete set along the genome. Open circles are experimental data and the red dashed line is the fit.



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Appendix 1 Figure 8. Modeling experimental data with MM4 model in the case where the potential origins form a discrete set along the genome. Open circles are experimental data and the red dashed line is the fit.

	Continuous	Discrete	Continuous	Discrete
model	GoF_{global}	GoF_{global}	$(y_{exp} - y_{fit})^2$	$(y_{exp} - y_{fit})^2$
MM1	-0.95	-5.28	0.66	0.56
MM2	0.85	0.72	0.08	0.10
MM3	0.87	0.88	0.08	0.09
MM4	0.90	0.92	0.08	0.05

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Appendix 1 Table 1. Values of GoF_{global} and fitting residual norm $(y_{exp} - y_{fit})^2$ for each model.

Models comparison

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To address whether the better data fit with MM4 is solely due to the higher degree of complexity of the model, we used two different approaches : a traditional statistical hypothesis testing: the extra sum of squares F test (*Bevington and Robinson, 2003*) and the Akaike's criterion (ΔAIC) that is based on information theory (*Ljung, 1998*). We can objectively reject MM1 as it did not reproduce in a satisfactory manner the averaged fork density, $I(f)$ and eye-to-eye distances distributions (Appendix 1, *Figure 1* and *Figure 5*). MM2 and MM3 satisfactorily reproduced all measured quantities (Appendix 1, *Figure 2*, *Figure 3*, *Figure 6* and *Figure 7*) but with lower GoF_{global} value than the MM4 models (Appendix1, *Table 1*). The discrete MM4 model has higher GoF_{global} value than the continuous one, whereas the continuous MM2 and MM3 models were better than or equal to their discrete version, respectively (Appendix1, *Table 1*). To choose the best model, we compared the discrete MM4 model, continuous MM2, MM3 and MM4 corresponding to fits with highest GoF_{global} values (Appendix1, *Table 1*). Comparing the discrete MM4 with the continuous MM2, MM3 and MM4 models led in all cases to $F > 1$ with p-values $p < 10^{-6}$ and negative ΔAIC values (Appendix1, *Table 2*). The discrete MM4 model is therefore the best model and the observed increase in GoF_{global} does not reflect an overfitting of the data.

	model	F	p	ΔAIC
638	Continuous MM2	19.3	1.5×10^{-7}	-30.2
	Continuous MM3	16.9	8.3×10^{-7}	-26.6
	Continuous MM4	∞	Not defined	-31.1

639 **Appendix 1 Table 2.** Values of F-test, the associated p -value (p) and the ΔAIC when the discrete MM4
640 model is compared with continuous MM2, MM3 and MM4 model.

642 Appendix 2

643 The MM5 model used to generate the *in silico* data

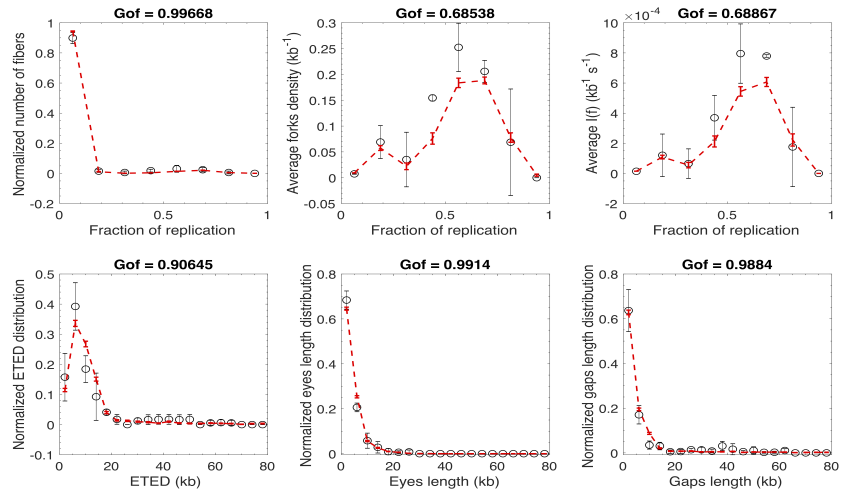
644 In MM5, localized potential origins were distributed with a uniform density $\rho = 1 \text{ kb}^{-1}$ and
645 N_{dom} domains of size l_{dom} were randomly positioned along a genome of length $L = 10^5 \text{ kb}$.
646 As in previous works, we assumed that at the start to S phase N_0 limiting factors were
647 available for origin firing and their number, $N(t)$, increased during the course of S phase as
648 $N(t) = N_0 + Jt$, and that each factor was sequestered by new forks upon origin activation
649 and released and made available again for origin firing upon coalescence of converging
650 forks. Forks progressed at a constant velocity $v = 0.5 \text{ kb.min}^{-1}$. The probability of origin
651 firing by encounter with a limiting factor was higher inside the domains ($P_0 + P_{dom}$) than
652 outside them (P_0). In addition, origins outside but not inside the domains had a non-null
653 probability P_{inhib} of being inhibited. Two local effects were allowed to act within a distance
654 d_{fork} from active forks: P_0 was enhanced by P_{fork} and origin inhibition was relieved with a
655 probability $P_{deinhib}$. We simulated 300 complete S phases using the 10 parameter values
656 listed in Appendix 2, **Table 1**, and extracted snapshots at 8%, 19% and 53% global replicated
657 fractions. Each snapshot was considered as an independent sample and for each of them:
658 i) the genome was randomly cut following the molecule length distribution presented in
659 **Figure 6**, ii) the data were reshaped as described in material and methods to account for
660 the finite experimental resolution and iii) the distributions of $I(f)$, replicated fraction of
661 single fibres, global fork density, eye-to-eye distances, gap lengths and eye lengths were
662 determined.

Parameter	Value
N_0	107
$J(s^{-1})$	29
P_0	0.11
P_{inhib}	0.96
P_{fork}	0.28
$d(kb)$	94.91
N_{dom}	196
l_{dom}	192.39
$P_{deinhib}$	0.06
P_{dom}	0.73

665 **Appendix 2 Table 1.** Values of MM5's parameters. These values are chosen arbitrarily

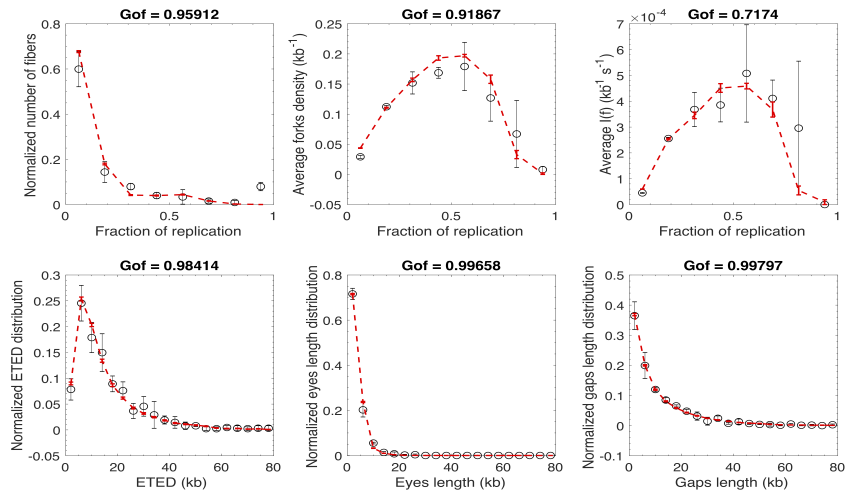
666 Fitting the *in silico* data by MM4 model

667 By independently fitting the simulated profiles of each global replicated fraction, we implicitly
668 assume that samples could originate from separated experiments, hence MM4 parameters
669 values are possibly different for each global replicated fraction. This allows us to accurately
670 reproduce observations from each sample (Appendix2 **Figure 1**, **Figure 2** and **Figure 3**).



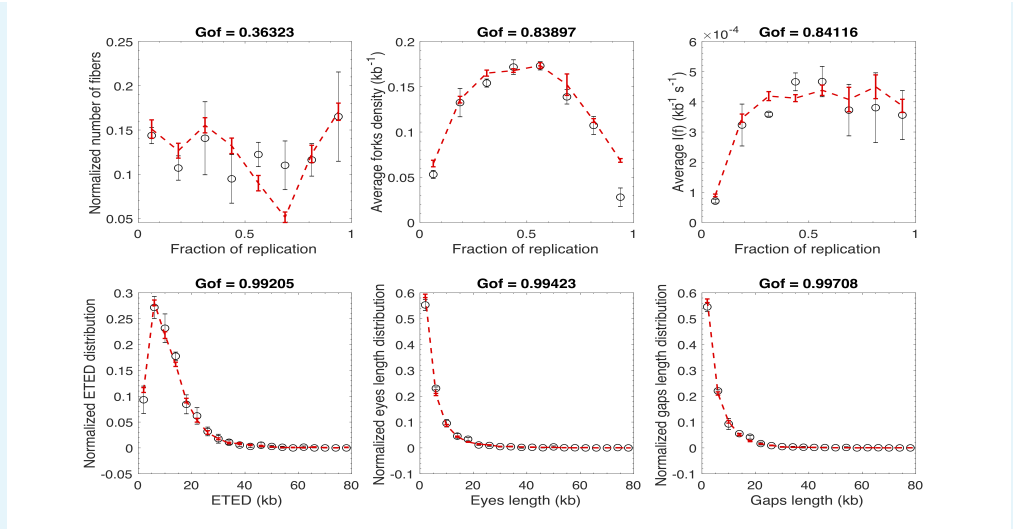
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Appendix 2 Figure 1. Modeling 8% global replicated fraction simulated data with discrete MM4 model. Open circles are simulated data and the red dashed line is the fit. $GoF_{global} = 0.96$



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Appendix 2 Figure 2. Modeling 19% global replicated fraction simulated data with discrete MM4 model. Open circles are simulated data and the red dashed line is the fit. $GoF_{global} = 0.97$



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Appendix 2 Figure 3. Modeling 53% global replicated fraction simulated data with discrete MM4 model. Open circles are simulated data and the red dashed line is the fit. $GoF_{global} = 0.82$

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Reduction of MM5 to MM4

In the MM5 model origins fire globally with two origin firing probabilities (P_0 and $P_0 + P_{dom}$) eventually increased by a local origin firing probability (P_{fork}) close to an active fork, and the genome is divided into domains that either support or escape some inhibitory probability of firing (assumed to represent inhibition by the intra-S checkpoint). As the position of these domains is not identical between repeated simulations, we can reduce their description by specifying a fraction θ ($\theta = \frac{N_{dom} l_{dom}}{L}$) of the genome where origins escape checkpoint inhibition. In these domains, the global origin firing probability $P_{in} = \frac{1}{2} (P_0 + P_{dom})$, with the $\frac{1}{2}$ pre-factor being due to normalization considerations. The local probability of origin firing (close to a fork) inside a domain is $P_{local}^{in} = \frac{1}{2} (P_0 + P_{dom} + P_{fork})$. Outside these domains, the global probability of origin firing is modulated by the probability of origin inhibition $P_{out} = \frac{1}{2} P_0 (1 - P_{inhib})$. In the same manner the local probability of origin firing is modulated by the action of intra-S checkpoint and the local cancellation of inhibition process $P_{local}^{out} = \frac{1}{2} (P_0 + P_{fork}) [1 + P_{inhib} (P_{deinhib} - 1)]$. Local probabilities of origin firing only influence origins over a distance d_{fork} downstream of a fork. The MM4 model contains a unique local probability of origin firing, that corresponds to the average value of the two local probabilities of origin firing, $P_{local} = \theta P_{local}^{in} + (1 - \theta) P_{local}^{out}$. Therefore, by considering the essential ingredients of the MM5 model, we combined the parameters of the model to retrieve the parameters of MM4 (**Table 2**).

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MM4	equivalence with MM5
N_0	N_0
J (s^{-1})	J
θ	$\frac{N_{dom} l_{dom}}{L}$
P_{in}	$\frac{1}{2} (P_0 + P_{dom})$
P_{local}	$\frac{1}{2} (P_0 + P_{fork}) [1 + (1 - \theta) P_{inhib} (P_{deinhib} - 1)] + \theta P_{dom}$
P_{out}	$\frac{1}{2} P_0 (1 - P_{inhib})$
d (kb)	d

Appendix 2 Table 2. Reducing MM5 to MM4.

The values of these parameters can be compared directly to parameters of MM4 model

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obtained from the fitting of the simulated data for each sample (**Table 3**). To assess if the difference between the expected and the inferred value of a parameter is statistically significant we calculate $t = \frac{(\text{expected value} - \text{inferred value})^2}{\text{error}^2}$, for $t \geq 1$ the difference is statistically significant otherwise it is not. The values of parameters changed as the global replicated fraction increased (Appendix 2, **Table 3**). To assess the level of significance of these variations we calculated $\chi^2 = \frac{(\text{parameter}_1 - \text{parameter}_2)^2}{\text{error}_1^2 + \text{error}_2^2}$ coefficient between the values of the same parameter obtained for different global replicated fraction. If $\chi^2 < 1$ the difference between the two values was not statistically significant otherwise it was significant. Appendix 2, **Figure 4** shows that the differences of predicted parameters values among the 3 considered samples were not statistically significant, as was expected.

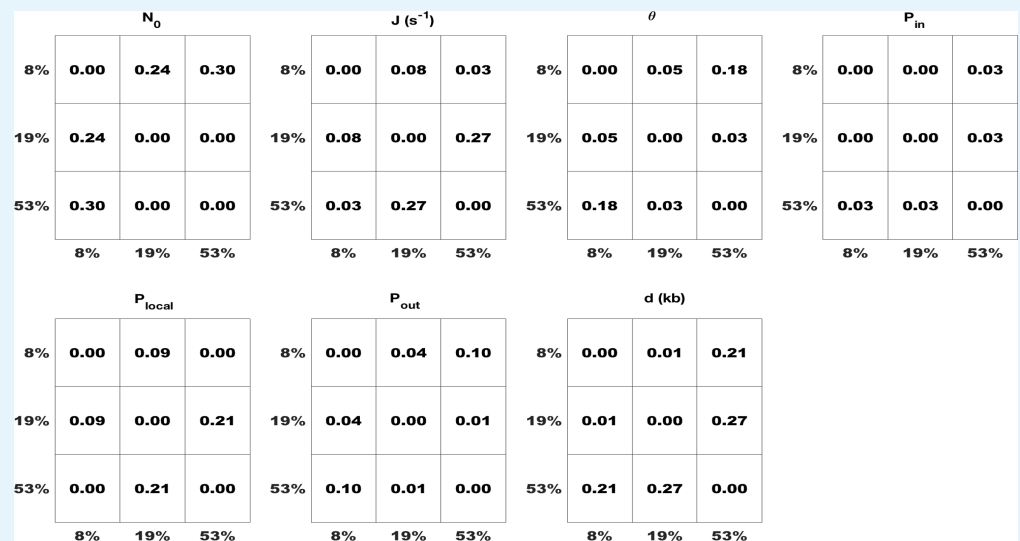
MM4	Input	8%	19%	53%
N_0	107	83.86 ± 32 ($t < 1$)	125 ± 29 ($t < 1$)	129 ± 26 ($t < 1$)
J (s^{-1})	29	43.6 ± 46 ($t < 1$)	17 ± 9 ($t < 1$)	27 ± 3.4 ($t < 1$)
θ	0.38	0.25 ± 0.2 ($t < 1$)	0.35 ± 0.16 ($t < 1$)	0.42 ± 0.1 ($t < 1$)
P_{in}	0.42	0.4 ± 0.2 ($t < 1$)	0.41 ± 0.17 ($t < 1$)	0.5 ± 0.2 ($t < 1$)
P_{local}	0.22	0.23 ± 0.09 ($t < 1$)	0.17 ± 0.05 ($t < 1$)	0.23 ± 0.04 ($t < 1$)
P_{out} ($\times 10^{-3}$)	2.2	1.1 ± 1 ($t < 1$)	1.9 ± 1 ($t < 1$)	2.3 ± 1 ($t < 1$)
d (kb)	94.91.	135 ± 86 ($t < 1$)	119 ± 57 ($t < 1$)	51 ± 32 ($t < 1$)

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Appendix 2 Table 3. Comparison between the expected and inferred values of MM4 parameters.

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All $t < 1$ and $\chi^2 < 1$ (Appendix 2, **Figure 4**), meaning the constancy of parameters values for all three samples. Therefore, we conclude that the optimization procedure was able to circumscribe the expected parameters values in an accurate manner for each sample. It should be noted that we choose a very conservative criterion to assess if two parameters are different or not. The conditions of $\chi^2 = 1$ or $t = 1$ are equivalent to a confidence level of $\alpha = 10^{-7}$ in the case of a two sided and one sided t statistics. In other words, with our criterion the probability to find that the values of two parameters are different by chance is smaller than 10^{-7} .



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Appendix 2 Figure 4. The values of each MM4 model parameter were compared pair-wise between samples with different global replicated fraction. The statistical significance of their difference was assessed by χ^2 test and represented as a binary heat map where not statistically significant differences are coloured in white and statistically significant difference are coloured in blue. The number in each box is the χ^2 coefficient.

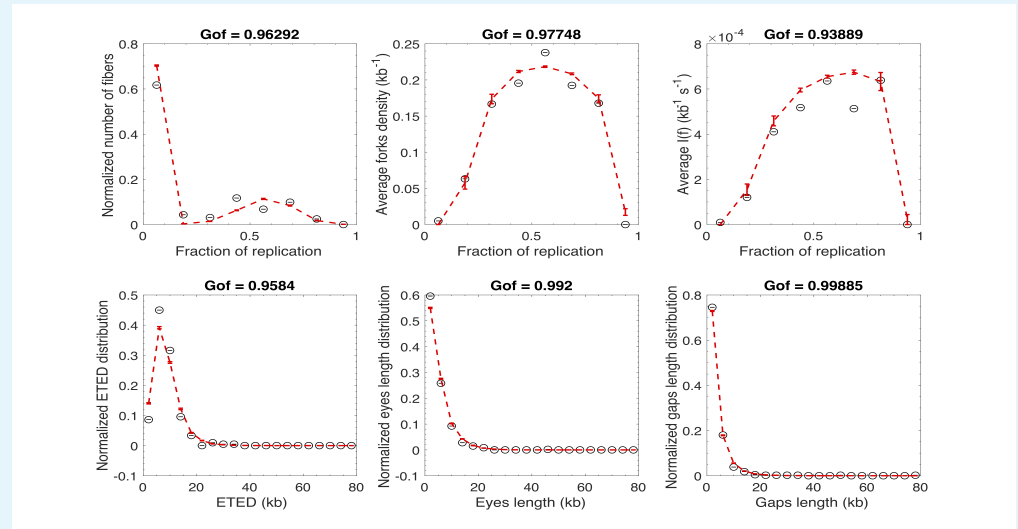
734 The ability of the fitting procedure i) to circumscribe the values of MM4 model parameters
735 close to the expected ones (Appendix 2, **Table 3**) and ii) to retrieve the constancy of these
736 parameter's values as the global degree of replication increases (Appendix 2, **Figure 4**)
737 demonstrates the adequacy of our fitting strategy to recover the dynamic of DNA replication
738 during S phase in the framework of MM4 model by setting the null hypothesis as : the
739 values of MM4 parameters do not change as S phase progresses. Therefore, rejection of this
740 hypothesis for a considered parameter means its variation during S phase.

741 **Appendix 3**

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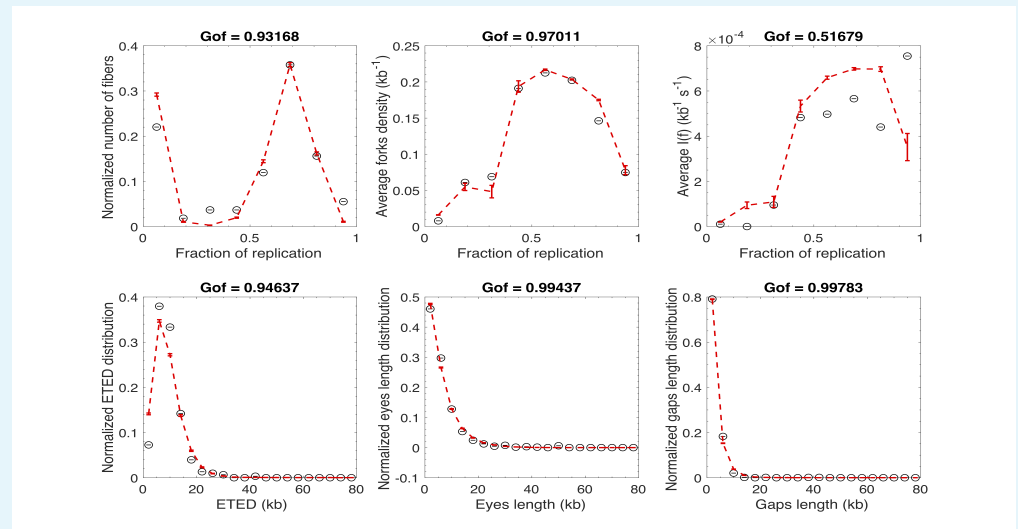
Fitting the experimental profiles by MM4 model : Unchallenged S phase

We fitted independently the measured profiles for each global replicated fraction by discrete MM4 model. The fits of observations from 8% global replicated fraction are presented in Appendix 1 **Figure 8** and those of 19% and 53% are presented Appendix 3 **Figure 1** and **Figure 2** respectively. In Appendix 3 **Table 1** we give the value of the fitted parameters. The reliability of observed differences among inferred MM4 parameters are assessed statistically by using χ^2 coefficient as defined in Appendix 2 (Appendix 3 **Figure 3**)



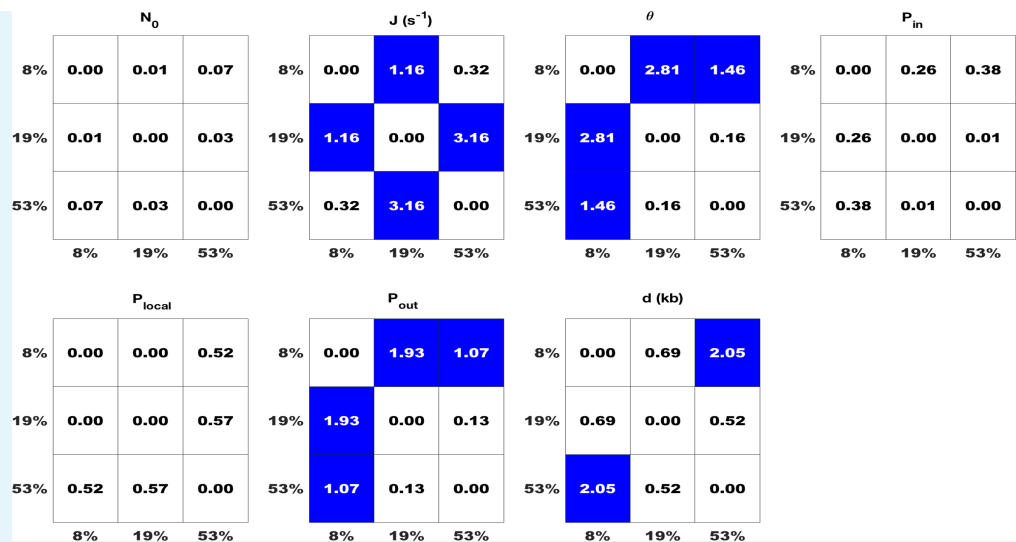
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Appendix 3 Figure 1. Modeling measured sample with 19% global replicated fraction with the discrete MM4 model. Open circles are simulated data and the red dashed line is the fit. $GoF_{global} = 0.96$



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Appendix 3 Figure 2. Modeling measured sample with 53% global replicated fraction with the discrete MM4 model. Open circles are simulated data and the red dashed line is the fit. $GoF_{global} = 0.90$



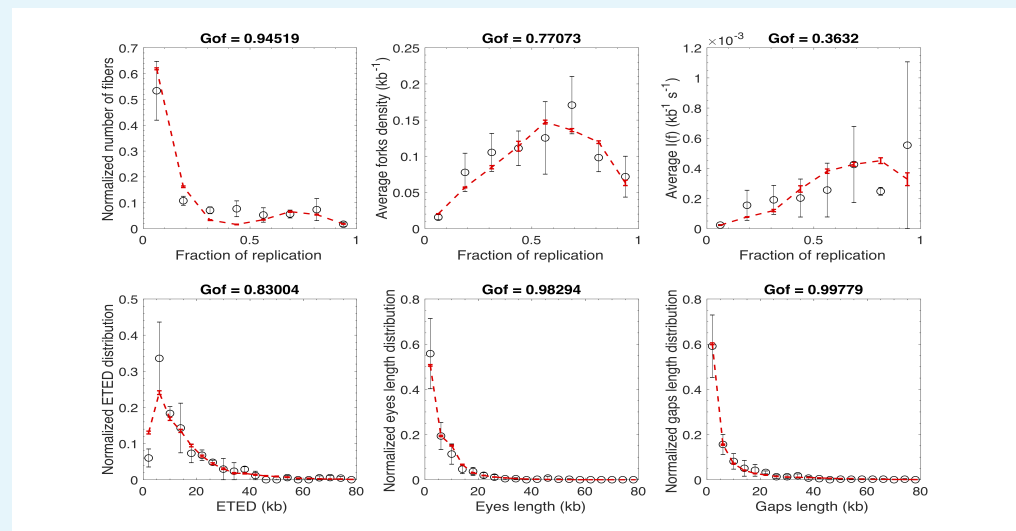
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Appendix 3 Figure 3. The values of each MM4 model parameter were compared pair-wise between samples with different global replicated fraction. The statistical significance of their difference was assessed by χ^2 test and represented as a binary heat map where the white colour represents no statistically significant difference and the blue colour represents statistically significant difference. The number in each box is the χ^2 coefficient.

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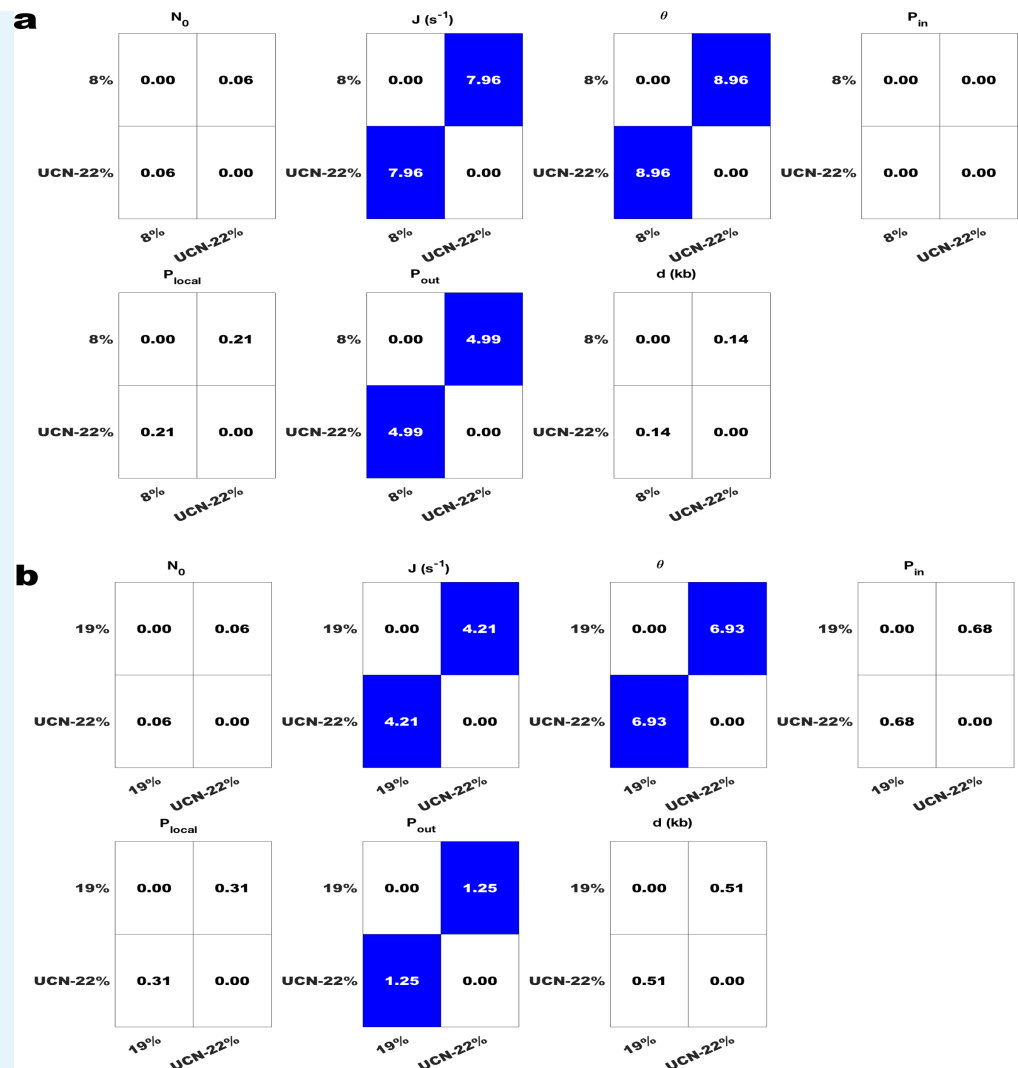
Fitting the experimental profiles by MM4 model : Chk1 inhibited S phase

We fitted with the discrete MM4 model a sample that had spent in the presence of UCN-01 the same time interval in S phase as the control sample with 8% global replicated fraction. The global replicated fraction of the of the UCN-01 sample was 22%. The fits are presented in Appendix 3 **Figure 4** and the obtained parameters values are given in Appendix 3 **Table 1**. The reliability of observed differences among inferred MM4 parameters between controls and Chk1 inhibited sample are assessed statistically by using χ^2 coefficient as defined in Appendix 2 (Appendix 3 **Figure 5**)



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Appendix 3 Figure 4. Modeling a measured sample with 22% global replicated fraction in presence of UCN-01 with discrete MM4 model. Open circles are simulated data and the red dashed line is the fit. $GoF_{global} = 0.85$



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Appendix 3 Figure 5. a. Comparing samples that have spent the same time interval in S phase. **b.** Comparing samples that have similar global replication fractions. The values of each MM4 model parameter were compared pair-wise between samples with different global replicated fraction. The statistical significance of their difference was assessed by χ^2 test and represented as a binary heat map where the white colour represents no statistically significant difference and the blue colour represents statistically significant difference. The number in each box is the χ^2 coefficient.

MM4	unchallenged: 8%	unchallenged: 19%	unchallenged: 53%	UCN-01: 22%
N_0	1064 ± 135	1043 ± 116	1002 ± 106	1006 ± 102
$J (s^{-1})$	601 ± 198	1026 ± 196	404 ± 151	1467 ± 89
θ	0.25 ± 0.06	0.43 ± 0.04	0.39 ± 0.05	0.56 ± 0.032
P_{in}	0.41 ± 0.07	0.34 ± 0.07	0.32 ± 0.07	0.42 ± 0.07
P_{local}	0.43 ± 0.06	0.43 ± 0.06	0.52 ± 0.06	0.38 ± 0.06
P_{out}	0.09 ± 0.02	0.17 ± 0.04	0.15 ± 0.03	0.23 ± 0.04
$d (kb)$	143.8 ± 36.3	91.5 ± 25.6	56.1 ± 23.6	119.3 ± 29.3

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Appendix 3 Table 1. Values and the corresponding errors of MM4 parameters for the best fit of each sample and each condition.