Kinetic Model of DNA Replication in Eukaryotic Organisms

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We formulate a kinetic model of DNA replication that quantitatively describes recent results on DNA replication in the system of

on replicated and unreplicated regions. The experi-

replicating at the same time, then the distributions would have been concentrated over a very small range of . But, as one can see in Figure 3(c),

are quite good, except where the finite size of the combed DNA fragments becomes relevant. For example, when mean hole, eye, and eye-to-eye lengths exceed about 10% of the mean fragment size, larger segments in the distribution for $\ell_h(\ )$, etc., are excluded and the averages are biased down. We confirmed this with the Monte-Carlo simulations, the results of which are overlaid on the experimental data. The finite fragment size in the simulation matches that of the experiment, leading to the same downward bias. In Figure 5, we overlay the fits on the experimental data. We emphasize that we obtain ( ) directly from the data, with no fit parameters, apart from an overall scaling of the time axis. The analytical form is just a model that summarizes the main features of the origin-initiation rate we determine a our model, from the experimental data. The important result is ( ).

From the maximum of $_{tot}(\ )$, we find a mean spacing between activated origins of 6.3($\pm$0.3) kb, which is much smaller than the minimum mean eye-to-eye separation 14.4($\pm$1.5) kb. In our model, the two quantities differ if initiation takes place throughout S-phase, as coalescence of replicated regions leads to fewer domains, and hence fewer inferred origins (see the note below equation (5)). The mean eye-to-eye separation is of particular

Discussion

Initiation throughout S-phase

The view that we are led to here, of random initiation events occurring continuously during the replication of *X. laevis* sperm chromatin in egg extracts, is in striking contrast to what has until recently been the accepted view of a regular periodic organization of replication origins throughout the genome.[8,9,30,31] For a discussion of experiments that raise doubts on such a view, see Berezney *et al.*[32] The application of our model to the results of Herrick *et al.* indicates that the kinetics of DNA replication in the *X. laevis* system closely resembles that of genome duplication in early embryos. Specifically, we find that the time required to duplicate the genome agrees well with what is observed. In addition, the model yields accurate values for replicon sizes and replication fork velocities that confirm previous observations.[7,28] Though replication may differ biologically from what occurs, the results nevertheless demonstrate that the kinetics remains essentially the same. Of course, the specific finding of an increasing rate of initiation invites a biological interpretation involving a kind of autocatalysis, whereby the replication process itself leads to the release of a factor whose concentration determines the rate of initiation. This will be explored in future work.

Directions for future experiments in *X. laevis*

One effect that we did not include in our analysis is a variable fork velocity. For example, might decrease as forks coalesce or as replication factor becomes limiting toward the end of S-phase.[5,22–24] Such effects, if present, are too small to see in the data analyzed here.

Another important question is to separate the effects of any intrinsic distribution due to early and late-replicating regions of the genome of a single cell from the extrinsic distribution caused by having many cells in the experiment. One approach would be to isolate and comb the DNA from a single cell. Although difficult, such an experiment is technically feasible. The latter problem could be resolved by fluorescence observations of the chosen cell.

Applications to other systems

One can entertain many further applications of the basic model discussed above, which can be generalized, if need be. For example, Blumenthal *et al.* interpreted their results on replication in for to imply periodically spaced origins in the genome[21] (see their Figure 7). It is difficult to judge whether their peaks are real or statistical happenstance, but if the conclusion is indeed that the origins in that system are arranged periodically, the kinetics model could be generalized in a straightforward way (introducing an (,) that was periodic in).

Very recently, detailed data on the replication of budding yeast ( ) have become available.[33] The data provide information on the locations of origins and the timings of their initiation during S-phase. These data support the view of origin initiation throughout S-phase. Unlike replication in *X. laevis* prior to the mid-blastula transition, origins in budding yeast are associated with highly conserved sequence elements (autonomous replication sequence elements, or ARSs). Raghuraman *et al.*[33] also give the first estimates of the distribution of fork velocities during replication. Although broad, the distribution is apparently stationary, and there is no correlation between velocities and the time in S-phase when the forks are initiated. The model developed here could be generalized in a straightforward way to the case of budding yeast. Knowing the sequence of the genome and hence the location of potential origins means that the initiation function would be an explicit function of position along the genome, with peaks of varying heights at each potential origin. The advantage of the kind of modeling advanced here would be the opportunity to derive quantities such as the replication fraction as a function of time in S-phase. Raghuraman *et al.* fit their data for this "timing curve" to an arbitrarily chosen sigmoidal function (see their supplementary data, Section

raises other issues. First, it requires an unknown mechanism to achieve this periodicity of POR spacing. Second, it assumes implicitly that most of the PORs fire during S-phase, to prevent the 30 kb gap that could arise from an origin's failure to

combing process. The lattice is then “coarse grained” by averaging over approximately four pixels. The coarse lattice length scale is then 0.24 μm, which roughly corresponds to that of the scanned optical images. Finally, the coarse-grained fragments were analyzed to compile statistics concerning replicon sizes, eye-to-eye sizes, etc., that were directly compared to experimental data.

In a first version of the simulation, the lattice was directly simulated using a vector with one element for each lattice site. In a more refined version of the simulation, we noted only the position of the replication forks, which greatly increased the speed of the simulations.

We also used the simulation to test a previous algorithm for extracting ( ), the initiation rate as a function of overall replication fraction. The previous algorithm[13,47] looked for small replicated regions and extrapolated back to an assumed initiation time. We tested this algorithm using our Monte-Carlo analysis and found significant bias in the inferred ( ), while the algorithms we introduce here showed no such bias.

Parameter extraction from data

We extracted data from both the real experiments and the Monte-Carlo simulations by a global least-squares fit that took into account simultaneously the different data collected (i.e. the different curves in Figures 3 and 4). As

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