Replicon-seq

Polo Rivera, Cristian; Deegan, Tom D.

Published in:
Trends in Genetics

DOI:
10.1016/j.tig.2022.05.012

Publication date:
2022

Trends in Genetics

CellPress OPEN ACCESS

Spotlight

Replicon-seq: seeing is believing

Cristian Polo Rivera [1] and Tom D. Deegan [2,*]

Claussin *et al.* introduce Replicon-seq, a new genome-wide DNA sequencing technology that monitors the progression of individual replisomes at high resolution *in vivo*.

Accurate DNA replication is essential for the faithful transmission of genetic information during cell division and is performed by a complex macromolecular machine called the replisome. Our molecular understanding of DNA replication in eukaryotes has exploded over the past decade, driven by bulk and single-molecule biochemical systems that recapitulate DNA replication *in vitro*, the cryo-electron microscopy (cryo-EM) revolution, and technological advances in DNA sequencing technologies which facilitate the study of DNA replication *in vivo*. Despite this recent progress, many currently available genomic methods for studying DNA replication *in vivo* rely on population-based DNA sequencing and are therefore of limited use for high-resolution studies of individual replisomes. Recently, several single-molecule DNA sequencing approaches have been developed to overcome these limitations [1,2]. Claussin *et al.* describe an elegant new example of one such technology, Replicon-seq, that permits the study of individual replisomes at single-nucleotide resolution in budding yeast cells [3]. Adapting the technology of chromatin endogenous cleavage combined with deep sequencing (ChEC-seq) [4], the authors fused micrococcal nuclease (MNase) to the Mcm4 subunit of the Cdc45–MCM–GINS (CMG) replicative helicase which forms the core of the eukaryotic replisome. After harvesting the yeast cells, the Mcm4–MNase fusion protein is rapidly activated in the presence of calcium, leading to CMG-proximal DNA cleavage. Cleavage at two sister replisomes that have emanated from a single DNA replication origin liberates an entire replicon, which can then be sequenced in its entirety using Nanopore sequencing, and the nascent DNA molecules (marked with the thymidine analogue bromodeoxyuridine, BrdU) are selected *in silico*. The alignment of multiple sequencing reads from a specific genomic location generates a so-called tornado plot from which one can infer the dynamics of replisome progression in that region (Figure 1).

The majority of tornado plots are highly symmetrical and are centred on known DNA replication origins, demonstrating that, in general, divergent sister replisomes travel at a consistent speed independently of the local chromatin environment, indicative of robust mechanisms to ensure smooth replisome progression. The authors are, however, able to detect some replisome stalling/pausing events in wild-type cells. The stalling of one replisome at the well-characterised proteinaceous Fob1 barrier does not impact upon the progression of the sister replisome, suggesting a lack of physical coupling or coordination between divergent sister replisomes, largely in agreement with previous studies [5]. Replisome pausing is also detected at tRNA genes, centromeres, telomeres, and DNA replication origins. Notably, the detected replisome pausing events are transient, lasting less than 1 minute, before replisome progression continues downstream of the stall site. The duration of replisome pausing at tRNA genes and centromeres was extended fourfold in cells that lacked the accessory helicase Rrm3, consistent with previous work [6].

Replicon-seq also allowed the authors to detect replisome pausing at 208 genes but, strikingly, replication–transcription conflicts are mitigated quickly in wild-type cells. Replisomes slow momentarily at the promoter regions of some (but not all) highly transcribed genes (e.g., *PDC1*), but no general correlation is observed between the levels of S-phase transcription and the extent of replisome pausing in genic regions. Rrm3 helicase again supports smooth replisome progression at highly transcribed genes, aiding replisome progression past the RNA polymerase II preinitiation complex in these regions.

The authors also use Replicon-seq to monitor DNA replication termination, represented by the merging of sequencing reads emanating from neighbouring replication origins. Previous analyses of replication termination have been largely limited to studies of plasmid replication, either in biochemically reconstituted *in vitro* systems or on episomes in budding yeast [7,8]. However, Replicon-seq affords the key advantage of studying replication termination on replicating chromosomes *in vivo*. Interestingly, the authors describe a role for the accessory helicase Rrm3 in supporting efficient replication termination on chromosomes, even in the absence of an intervening protein barrier, in agreement with previous experiments on plasmids [6,7]. Taken together, these data (and those described in the preceding text) confirm the crucial role for Rrm3 in supporting efficient DNA replication *in vivo*.

Going forward, Replicon-seq provides an exciting technological platform for precisely monitoring replisome progression *in vivo*, and should complement and synergise with extensive molecular studies already being carried out using biochemical and cryo-EM approaches in the DNA replication field. The ease of genetic manipulation in budding yeast should also allow examination of the dependencies and mechanisms of replisome progression at specific loci. For example, a multitude of factors, such as RNase H and Sen1, have been ascribed a role in resolving replication–transcription collisions [9]; Replicon-seq provides a powerful system

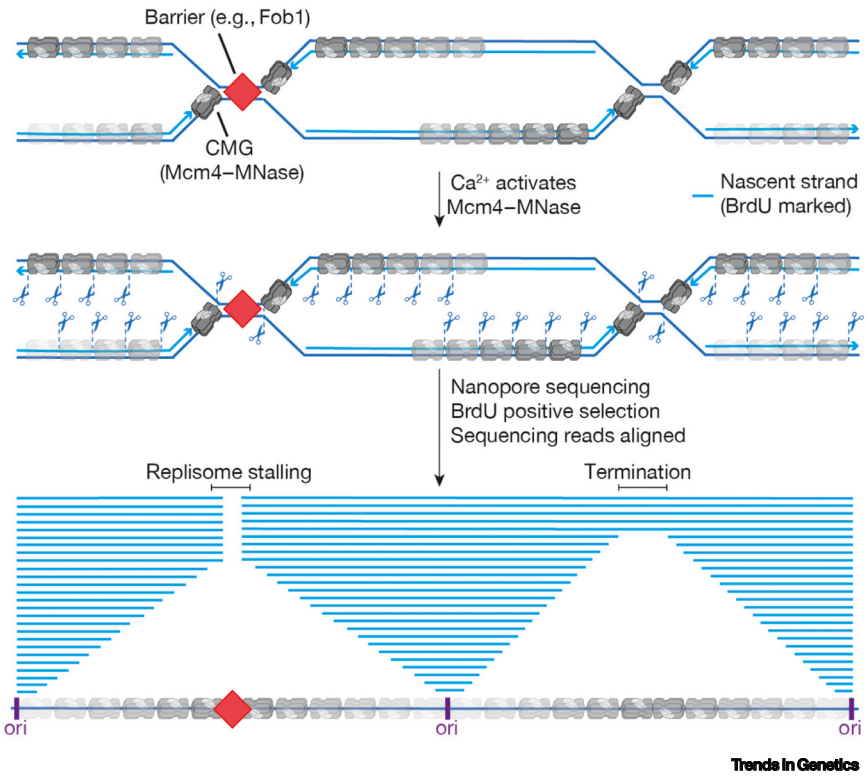

Figure 1. Schematic of Replicon-seq. Budding yeast cells harbouring an Mcm4–MNase fusion are released synchronously into S-phase in the presence of BrdU. Calcium-dependent activation of MNase following cell harvesting triggers CMG-proximal DNA cleavage, and nascent DNA molecules encompassing entire replicons are then sequenced via Nanopore sequencing. This approach allows genome-wide mapping of DNA replication, including initiation, elongation, replisome stalling, and termination, at single-nucleotide resolution. Replisomes are depicted as CMG only (multiple replisome components have been omitted for simplicity). Abbreviations: BrdU, bromodeoxyuridine; CMG, Cdc45–MCM–GINS replicative helicase; ori, origin of replication; MNase, micrococcal nuclease.

to interrogate these events genome-wide and at high resolution. Replicon-seq might also be adapted to investigate how replisomes behave under conditions of DNA replication stress or upon meeting site-specific DNA lesions. Some DNA lesions, such as DNA interstrand crosslinks and single-stranded DNA nicks, can trigger the disassembly or dissociation of CMG from DNA, and inhibition of DNA polymerases (e.g., in aphidicolin-treated cells) can cause physical uncoupling of CMG from the leading strand DNA polymerase ε [10]. Perhaps MNase-tagging of other replisome components for Replicon-seq analyses could be informative in such circumstances.

In the longer term, the adaptation of Replicon-seq for the study of DNA replication in other eukaryotes with larger and more complex genomes will be interesting. Notably, mammalian cells have less well defined DNA replication origins than budding yeast, and spatiotemporal patterns of DNA replication can thus vary significantly between different cells and between different S-phases [11]. In theory, this heterogeneity could limit the ability to map replisome progression by comparing different sequencing reads generated by Replicon-seq. Equally, the current limits on read length in Nanopore sequencing could be problematic in mammalian cells with long replicons. However, the rapid advances in long-read DNA sequencing technologies could soon provide a solution to this problem. In any case, Replicon-seq promises to be a powerful tool for future studies of DNA replication at high resolution *in vivo*.

Acknowledgments

Work in the laboratory of T.D.D. is supported by MRC University Unit funding to the MRC Human Genetics Unit. C.P.R. holds a PhD studentship funded by a Cancer Research UK (CRUK) Programme Grant (C578/A25669) to Karim Labib (MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee).

Declaration of interests

The authors declare no conflicts of interest.

[1]Medical Research Council (MRC) Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK
[2]MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK

*Correspondence:
tdeegan@ed.ac.uk (T.D. Deegan).

https://doi.org/10.1016/j.tig.2022.05.012

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