MOLECULAR & CELLULAR ONCOLOGY
2021, VOL. 8, NO. 2, e1839294 (3 pages)
https://doi.org/10.1080/23723556.2020.1839294

Taylor & Francis
Taylor & Francis Group

AUTHOR'S VIEWS

Control of S phase duration: a replication capacity model with E2F transcription at its heart

Cosetta Bertoli [a] and Robertus A.M. de Bruin [a,b]

[a]MRC Laboratory for Molecular Cell Biology, University College London, London, UK; [b]UCL Cancer Institute, University College London, London, UK

ABSTRACT

DNA replication capacity, the maximal amount of DNA a cell can synthesize at any given time during S phase, is controlled by E2F-dependent transcription. Controlling replication capacity limits the replication rate and provides a robust mechanism to keep replication fork speed within an optimal range whilst ensuring timely completion of genome duplication.

ARTICLE HISTORY
Received 11 August 2020
Revised 10 October 2020
Accepted 13 October 2020

KEYWORDS
DNA replication; S phase; DNA replication capacity; E2F-dependent transcription; genome stability

Introduction

Accurate genome duplication is essential for life. During the cell cycle, a number of regulatory mechanisms ensure that the genome is duplicated completely, accurately, and only once per cell cycle during the synthesis phase (S phase) before it is segregated during mitosis to produce two genetically identical daughter cells. Upon S phase entry DNA replication is initiated, known as firing, from discrete location in the genome, termed replication origins. Replication proteins bind to origins in a highly regulated manner, which is known as licensing. Firing allows for replication to be initiated from replication origins forming so-called replication bubbles, where two replication forks emanating from an origin proceed in opposite directions. The number of origins that are licensed is much higher than the actual number of origins that fire, leaving the majority of origins in an inactive, dormant state.[1] This allows for plasticity during the DNA replication process; if a replication fork stalls, another dormant origin nearby becomes active and replication can be completed. In addition, not all active origin fire at the beginning of S phase but follow a spatial and temporal firing program with early, mid, and late firing origins. This program is linked to the three-dimensional organization of the genome that is in turn linked to the structure and function of chromatin. This ensures that only a limited number of the active replication origins fire at a certain time during S phase.[1]

Replication capacity determines the length of S phase

The total amount of DNA being synthesized at a given time during S phase is determined by the number of active replication forks and the speed at which these travel. This is the DNA synthesis rate, which determines the time it takes to duplicate the entire genome and thereby S phase duration. Empiric observations suggest that a reduction in the firing of origins is compensated for by an increase in replication fork speed and *vice versa*.[2–4] This inverse relationship between the amount of firing and the speed of replication forks supports the idea that there is a limited amount of DNA a cell can synthesis per unit time.

In our recently published work,[5] where we explore this concept, we propose a replication capacity model, whereby cells have an intrinsic mechanism to limit the amount of DNA they can synthetize at any given time throughout S phase. We argue that a mechanism that limits the amount of DNA synthesis ensures timely completion of genome duplication independently of fluctuations in the number of active replication forks. This would ensure that replication fork speed is maintained within a safe range, preventing replication forks from traveling too slowly or too quickly (Figure 1a), which has been linked to the generation of genomic instability.[6]

E2F transcription determines replication capacity

Our work suggests that E2F-dependent transcription lies at the basis of the mechanism that controls replication capacity during S phase. Activation of E2F-dependent transcription drives the transition between Gap1 (G1) phase and S phase and is central to cell cycle entry. It is regulated by a family of transcription factors (E2F transcription factors 1–8) and their co-regulators, the pocket proteins, whose most prominent member is the protein Retinoblastoma 1, RB1. E2F-dependent genes encode for proteins required for cell cycle progression and DNA replication and repair.[7] Activation of E2F-dependent transcription in G1 is followed by its inactivation during S phase, giving rise to a wave of G1/S transcription, with target gene expression peaking at different times during the G1 and S phases.[8]

CONTACT Cosetta Bertoli c.bertoli@ucl.ac.uk MRC Laboratory for Molecular Cell Biology, University College London, Gower street, London, WC1E 6BT, UK

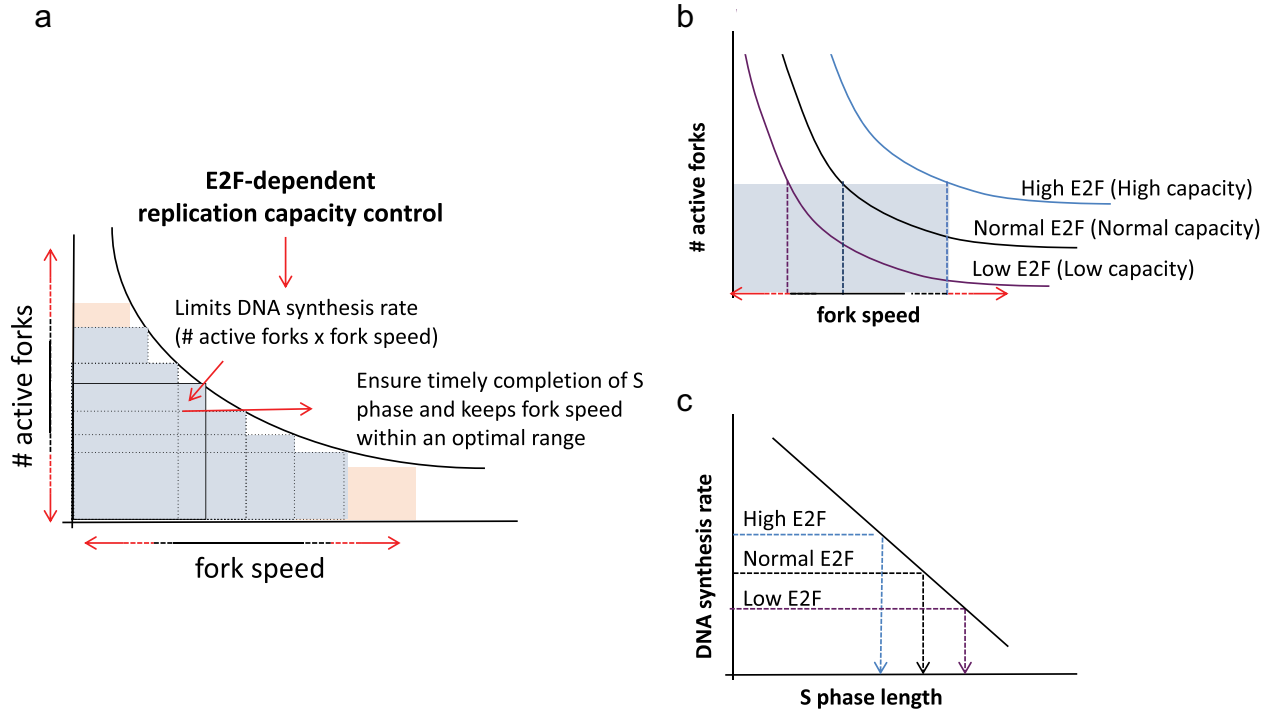

Figure 1. Molecular mechanism through which replication capacity regulates DNA replication rate and S phase length via E2F transcription. A. Replication rate (shaded areas) is given by the amount of active replication forks and the speed at which they travel. Replication capacity (black line) ensures that the area remains constant in case of variation in the number or speed of replication forks. E2F transcription sets replication capacity, therefore, replication rate remains relatively constant. B. Increasing E2F transcription increases replication capacity, extending replication rate (shaded area). It mostly affects replication fork speed. Conversely, decreasing E2F transcription decreases replication rate. C. Increasing replication rate, by increasing E2F transcription, decreases S phase duration.

Our work shows that preventing inactivation of E2F-dependent transcription during S phase, via depletion of the repressor E2F6, increases the amount of DNA cells can synthesis per unit time during S phase, thus shortening the S phase length. Conversely, overexpressing E2F6, by reducing E2F-dependent transcription, decreases DNA synthesis rates (Figure 1b, C). We propose that E2F-dependent transcription controls replication capacity by controlling the gene expression levels of replication factors required for DNA replication.

Our findings extend the central role of E2F-dependent transcription in controlling S phase. Our work establishes that in addition to driving S phase entry it also determines the S phase duration, which is in addition to its critical role in the cellular response to replication stress for maintenance of genome stability.[9]

Together these findings support a general role for E2F transcription in the S phase control and homeostasis, showing an overarching role for this transcriptional network in the mechanisms controlling genome duplication both in unperturbed conditions and under stress.

Importance for genomic stability and cancer

The concept that replication fork speed must be maintained within an optimal range is already well supported by the finding that significantly slowing down or speeding up replication forks increases fork stalling, and potentially collapse, with implications for genome stability.[6] A mechanism that provides a framework within which replication dynamics can fluctuate, by indirectly controlling replication fork progression to stay within an optimal range, is an important homeostatic mechanism for S phase. Our data show that increasing the speed of replication forks, by increasing replication capacity, causes cell cycle arrest and generates a certain level of DNA damage in subsequent cell cycles. It is not clear what causes the DNA damage, but it is possible that some under-replicated DNA or other unresolved structures are detected during mitosis and subsequently recognized by the checkpoint in the following G1. E2F-dependent transcription is often deregulated in cancer, as a consequence of oncogene activation, causing unscheduled S phase entry.[10] Our data suggest that besides shortening the G1 phase, deregulated E2F-dependent transcription is also likely to affect the replication capacity of cancer cells. Further research is required to establish if this provides a buffer to prevent oncogene-induced replication stress or contributes to the accumulation of genomic instability.

Funding

This work was supported by core funding to the MRC-UCL University Unit (Ref MC_U12266B) and funded by R.d.B.'s Cancer Research UK Programme Foundation Award CRUK CDF: C63833/A25729.

ORCID

Cosetta Bertoli http://orcid.org/0000-0001-5684-4630
Robertus A.M. de Bruin http://orcid.org/0000-0001-9957-1409

References

1. Fragkos M, Ganier O, Coulombe P, Méchali M. DNA replication origin activation in space and time. Nat Rev Mol Cell Biol. 2015;16:360–374.
2. Masai H, Matsumoto S, You Z, Yoshizawa-Sugata N, Oda M. Eukaryotic chromosome DNA replication: where, when, and how? Annu Rev Biochem. 2010;79(1):89–130. doi:10.1146/annurev.biochem.052308.103205.
3. Conti C, Saccà B, Herrick J, Lalou C, Pommier Y, Bensimon A. Replication fork velocities at adjacent replication origins are coordinately modified during DNA replication in human cells. Mol Biol Cell. 2007;18:3059–3067. doi:10.1091/mbc.E06-08-0689.
4. Anglana M, Apiou F, Bensimon A, Debatisse M. Dynamics of DNA replication in mammalian somatic cells: nucleotide pool modulates origin choice and interorigin spacing. Cell. 2003;114(3):385–394. doi:10.1016/S0092-8674(03)00569-5.
5. Pennycook BR, Vesela E, Peripolli S, Singh T, Barr AR, Bertoli C, de Bruin RAM. E2F-dependent transcription determines replication capacity and S phase length. Nat Commun. 2020;11. doi:10.1038/s41467-020-17146-z.
6. Merchut-Maya JM, Bartek J, Maya-Mendoza A. Regulation of replication fork speed: mechanisms and impact on genomic stability. DNA Repair (Amst). 2019;81:102654. doi:10.1016/j.dnarep.2019.102654.
7. Dimova DK, Dyson NJ. The E2F transcriptional network: old acquaintances with new faces. Oncogene. 2005;24(17):2810–2826. doi:10.1038/sj.onc.1208612.
8. Bertoli C, Skotheim JM, De Bruin RAM. Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol. 2013;14(8):518-28. doi:10.1038/nrm3629. PMID: 23877564; PMCID: PMC4569015..
9. Bertoli C, Herlihy AE, Pennycook BR, Kriston-Vizi J, De Bruin RAM. Sustained E2F-dependent transcription is a key mechanism to prevent replication-stress-induced DNA damage. Cell Rep. 2016;15:1412–1422. doi:10.1016/j.celrep.2016.04.036.
10. Kent LN, Leone G. The broken cycle: E2F dysfunction in cancer. Nat Rev Cancer. 2019;19:326–338. doi:10.1038/s41568-019-0143-7.