VISUALISING REPLICATION FORK TERMINATION WITH CLICK-CHEMISTRY-ENABLED SUPER-RESOLUTION MICROSCOPY

Darren A. Smith[1], Rebecca Jones[2], Agnieszka Gambus[2], Robert K. Neely[1,3]
[1]School of Chemistry, [2]Institute of Cancer and Genomic Sciences, [3]PSIBS Doctoral Training Centre, University of Birmingham, B15 2TT Birmingham, United Kingdom
Email: d.a.smith.3@bham.ac.uk

KEY WORDS: DNA, replication, termination, 5-hmC, single-molecule imaging, DNA combing

To ensure full coverage of the genome in a time-efficient manner, thousands of replication origins fire during eukaryotic DNA replication. The ensuing convergence of replication forks must be resolved through replication fork termination. In addition to replisome disassembly and disentanglement of nascent DNA, any DNA that remains between terminated forks must be replicated to maintain genome stability. A number of plausible mechanisms exist to explain replication fork termination, but these are generally speculative and the process remains poorly understood [1]. Since resolution of converged replication forks requires concerted structural rearrangement of a number of protein complexes, obtaining an estimate of the distance between forks when termination is first initiated is expected to provide useful mechanistic insight.

The distance between terminated replication forks can be estimated using fibre-FISH [2]. Control of polyubiquitylation (an essential process for replisome disassembly) allows the incorporation of 5-bromo-2'-deoxyuridine-5'-triphosphate (Br-dUTP) and digoxigenin-dUTP (DIG-dUTP) at specific points during replication. Subsequent staining by fluorescently-labelled antibodies, which preferentially bind to one or other of the nucleotide analogues, followed by molecular combing, allows direct visualisation of DNA replicated before and after termination. Unfortunately these measurements are severely hindered by the diffraction limit of light and, as such, cannot effectively discriminate between different possible models of replication termination. Super-resolution imaging offers the potential of greater insight; however, cross-adsorption of antibodies (which can be mitigated in bulk measurements) becomes particularly detrimental for the single-molecule localisation experiments required.

Here we circumvent antibody labelling by incorporating 5-hydroxymethylcytosine (5-hmC, a modified form of cytosine), rather than DIG-dUTP, during replication. 5-hmC is a target site for β-glucosyl transferase catalysed transfer of the β-D-glucosyl residue from uridine diphosphoglucose (UDP-Glu) [3]. Use of an azide-containing analogue of UDP-Glu provides a convenient platform from which to directly attach a fluorescent label through copper-free click-chemistry. Molecular combing of the labelled DNA on a hydrophobic surface produces linear DNA molecules that are in an ideal geometry to estimate the distance between terminated replication forks with super-resolution microscopy. While the results presented are specific to DNA replication termination, the labelling approach should be of value in other situations where incorporation of 5-hmC into a system can be controlled.

[1] R. Bailey, S. Priego Moreno, and A. Gambus, "Termination of DNA replication forks: 'Breaking up is hard to do'," *Nucleus*, **6**, 187–96 (2015)
[2] S. Priego Moreno, R. Bailey, N. Campion, S. Herron, and A. Gambus, "Polyubiquitylation drives replisome disassembly at the termination of DNA replication," *Science*, **346**, 477–81 (2014)
[3] T. Shahal, N. Gilat, Y. Michaeli, O. Redy-Keisar, D. Shabat, and Y. Ebenstein, "Spectroscopic quantification of 5-hydroxymethylcytosine in genomic DNA," *Anal. Chem.*, **86**, 8231–7 (2014)