Name	HASAN YARDIMCI
Position	Group leader (2nd 6)
Year joined *(Crick or founder institute)*	2013

Career History

1999 –2006 - PhD in Physics, Johns Hopkins University USA
2006–2008 - Post-doctoral Research Associate, University of Illinois Urbana Champaign USA
2008–2013 - Post-doctoral Research Associate, Harvard Medical School USA

Major Awards, Honours and Prizes

Membership of external committees, editorial boards, review panels, SABs etc

Lab Name	***Single Molecule Imaging of Genome Duplication and Maintenance Laboratory***

Research programme and achievements

In eukaryotes, prompt duplication of large genomes requires replication initiation at many sites, called origins. Each origin is 'licensed' for replication through co-ordinated assembly of inactive Mcm2-7 double hexamers onto double-stranded (ds) DNA, forming the pre-replication complex (pre-RC). Mcm2-7 hexamers are activated in S phase after association of Cdc45 and GINS. Cdc45-Mcm2-7-GINS (CMG) complex unwinds duplex DNA at the replication fork and acts as a hub to organise other replication factors around itself, thus assembling the replisome. Despite its importance as the foundation of the eukaryotic replisome, how the CMG helicase interacts with DNA at the replication fork and its dynamics at replication barriers have been controversial. My laboratory showed for the first time that recombinant purified CMG can efficiently bypass protein roadblocks on the lagging-strand template consistent with the steric exclusion model where the active helicase encircles single-stranded (ss) DNA in its central channel. Furthermore, by measuring DNA unwinding by individual CMG complexes with magnetic tweezers, we discovered that CMG unwinds DNA via a random walk, exhibiting both unwinding and backwards motion, with a significant propensity to pause. To elucidate the origin of frequent pausing by isolated CMG, we examined how DNA translocation by the helicase is regulated by its interaction with the replication fork. We discovered that the mechanistic basis for an order of magnitude slower duplex unwinding by CMG compared to its ssDNA translocation rate is engagement of the helicase with the parental duplex. Importantly, we found that association of ssDNA-binding-protein, RPA, with the excluded DNA strand

prevents duplex engagement by the helicase and speeds up the helicase at the fork. We further showed that backwards helicase motion is induced by DNA reannealing and can rescue a stalled helicase, serving a significant biological function. This “bottom-up” approach is allowing us to elucidate the dynamics of isolated CMG at the replication fork and to determine how other replisome components can mediate proper DNA engagement by the replicative helicase to achieve efficient fork progression.

The eukaryotic replisome navigates through a protein-rich chromatin environment and must overcome various protein obstacles including nucleosomes. To gain insight into how the replisome complex deals with nucleosomes, we examined DNA replication in *Xenopus laevis* egg extracts at the single-molecule level. Surprisingly, we found that the majority of parental histones are ejected from DNA upon replication fork arrival, with histone recycling, nucleosome sliding and replication fork stalling also occurring but at lower frequencies. We showed that expected local histone transfer only became dominant upon removal of free histones from extracts. Our studies provide the first direct evidence that parental histones remain in close proximity to their original loci during recycling and reveal that provision of excess histones results in impaired histone recycling, which has the potential to affect epigenetic memory. We are using the egg extract system as a “top-down” approach to illuminate the dynamics of pre-RCs and cohesin rings upon fork collision.

Research outputs

Burnham DR, Kose HB, Hoyle RB, Yardimci H. (2019) *The mechanism of DNA unwinding by the eukaryotic replicative helicase.* Nature Communications 10, 2159. DOI: 10.1038/s41467-019-09896-2
In this work, we monitored the movement of single CMG complexes in real time with high spatial and temporal resolution using magnetic tweezers. Our data showed that isolated CMG unwinds DNA one to two orders of magnitude slower compared to *in vivo* fork rates due to frequent helicase stalling at the replication fork. This is the first reported study to interrogate CMG dynamics at this level.

Kose HB, Larsen NB, Duxin JP, Yardimci H. (2019) *Dynamics of the eukaryotic replicative helicase at lagging-strand protein barriers support the steric exclusion model*. Cell Reports 26, 2113-2125. DOI: 10.1016/j.celrep.2019.01.086
Ring-shaped MCM hexamers initially load onto dsDNA at origins. However, whether the active CMG complex encircles dsDNA or ssDNA at the replication fork has been controversial. By analysing the outcome of CMG encountering strand-specific roadblocks, we showed that CMG encircles only one strand in its central channel during unwinding, which has important implications for the architecture of the eukaryotic replisome.

Eickhoff P, Kose HB, Martino F, Petojevic T, Abid Ali F, Locke J, Tamberg N, Nans A, Berger JM, Botchan MR, Yardimci H*, Costa A*. (2019) *Molecular Basis for ATP-Hydrolysis-Driven DNA Translocation by the CMG Helicase of the Eukaryotic Replisome*. Cell Reports 28, 2673–2688. *corresponding authors. DOI: 10.1016/j.celrep.2019.07.104
In this study, we used cryo-EM to image the eukaryotic replicative helicase as it performs ATP-hydrolysis-driven DNA translocation. We describe the molecular basis of fork unwinding and explain why not all sites around the ATPase hexamer are strictly required for translocation. We demonstrate for the first time that vertical movement of ssDNA through the

hexameric ATPase pore involves substrate rotation inside the helicase ring with a set of four subunits staircasing around ssDNA.

Gruszka D, Xie S, Kimura H, Yardimci H. (2020) *Single-molecule imaging reveals control of parental histone recycling by free histones during DNA replication*. Science Advances 6, 38. DOI: 10.1126/sciadv.abc0330
Parental histone recycling at the replication fork constitutes the mechanistic basis for epigenetic memory through cell division. The current consensus model suggests that most (if not all) parental histones are locally recycled during DNA replication. With single-molecule imaging, we unravel intricate histone dynamics and discover that, contrary to the prevailing view, not all histones are faithfully transferred onto daughter strands during replication. Importantly, we find that the efficiency of local histone recycling at the replication fork is a function of the free histone concentration.

Kose HB, Xie S, Cameron G, Strycharska MS, Yardimci H. (2020) *Duplex DNA engagement and RPA oppositely regulate the DNA-unwinding rate of CMG helicase*. Nature Communications 11, 3713. DOI: 10.1038/s41467-020-17443-7
The origin of relatively slow translocation rates of isolated replicative helicases and the mechanism by which other replication components increase helicase speed remained unclear. Here, we demonstrate that engagement of the eukaryotic CMG helicase with template DNA at the replication fork impairs its helicase activity, which is alleviated by RPA. Our work provides a mechanistic basis for relatively slow DNA unwinding by replicative helicases and explains how replisome components that interact with the excluded DNA strand can increase fork rates.