A New Assay Called NCAM Reveals Unexpected Checkpoint Kinase Function

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Chromosomal DNA is compacted to fit within a cell's nucleus in higher order nucleosome structures consisting of DNA wound around histone octamers and chromatin fibers. However, nucleosome structure is inhibitory to DNA-dependent processes including DNA replication and transcription. To overcome this nucleosome-based barrier, a variety of mechanisms exist that target histones (*e.g.*acetylation, displacement, and eviction), giving replication and transcription machinery access to DNA. Although mechanisms that promote transcription through nucleosomes are well studied, much less is known about how the replisome, a >2-MDa protein assembly that replicates DNA, traverses nucleosomes.

Post-doctoral fellow Jairo Rodriguez, with guidance from Toshio Tsukiyama in the Basic Sciences Division, set out to characterize the chromatin structure at replication forks in *S. cerevisiae*. This required a synchronous population of cells undergoing DNA replication (S-phase), which was achieved by hydroxyurea treatment to deplete dNTP levels and slow fork progression. High-density tiling microarrays covering chromosomes III, VI, and XII were used to map the DNA replication sites. 23 ORIs exhibited early firing and 18 ORIs fired late as evidenced by DNA copy profiles and chromatin immunoprecipitation (ChIP) of Pol I, a DNA polymerase subunit, to reveal fork position. Next, the authors determined whether nucleosome position and/or accessibility differed between S-phase and a non-replicating control (G1 phase) at these genomic regions. They were surprised to find that (1) nucleosome position did not change dramatically during fork progression and (2) nucleosome signal increased at forks. The authors were able to show that this phenomenon was not caused by an increase in nucleosome number. As a result, these data indicated that nucleosome accessibility had increased. This idea was confirmed by showing that MNase (a DNase that is normally unable to target nucleosomal DNA) cleaved S-phase replication fork chromatin more robustly than the non-replicating control at the few ORI's tested.

However, they still needed to devise a method to reveal replication-dependent nucleosome accessibility changes on a more *global* level.

Although they certainly could have used tiled microarrays to reveal the positions of more accessible nucleosomes (*i.e.* higher nucleosome signal), the data would be confounded by not knowing the histone density at the positions. To circumvent this problem, the authors devised the NCAM (normalized chromatin accessibility to MNase) assay in which nucleosome signals are normalized to histone H3 ChIP signals. This measurement gives the most accurate account of chromatin accessibility to MNase. Indeed, NCAM increased in S-phase cells at firing ORIs as expected but, intriguingly, NCAM extended ahead of Pol I and DNA synthesis regions. These data revealed an unanticipated increase in chromatin accessibility at and ahead of replication forks.

Since hydroxyurea treatment stalls replication forks and activates a cell-cycle checkpoint, Rodriguez wondered if checkpoint proteins contribute to the increased NCAM at replication forks. To test this, NCAM was measured for the checkpoint mutant *mec1*, an evolutionarily conserved kinase in the checkpoint response. NCAM had a 45% reduction in *mec1* cells compared to control, suggesting that Mec1 plays a role in promoting an open chromatin configuration at replication forks during the checkpoint response. They also present data showing that fork progression rate decreased in a *mec1* mutant during replication stress, indicating that Mec1-dependent increases in chromatin accessibility may facilitate DNA polymerase function during the repair process. Lastly, NCAM revealed increased chromatin accessibility at replication forks in cells undergoing normal S-phase (in other words, with no stress response) that was independent of Mec1.

Altogether, these results ascribe a new checkpoint-dependent role for Mec1 in promoting chromatin accessibility at replication forks. They also provide an invaluable tool, the NCAM assay, to gauge the degree of chromatin accessibility to MNase that is normalized to histone density. Future experiments will likely uncover Mec1 substrates at replication forks that promote open chromatin configurations during DNA replication.

Rodriguez J, Tsukiyama T. 2013. ATR-like kinase Mec1 facilitates both chromatin accessibility at DNA replication forks and replication fork progression during replication stress. *Genes Dev.* 27(1):74-86.

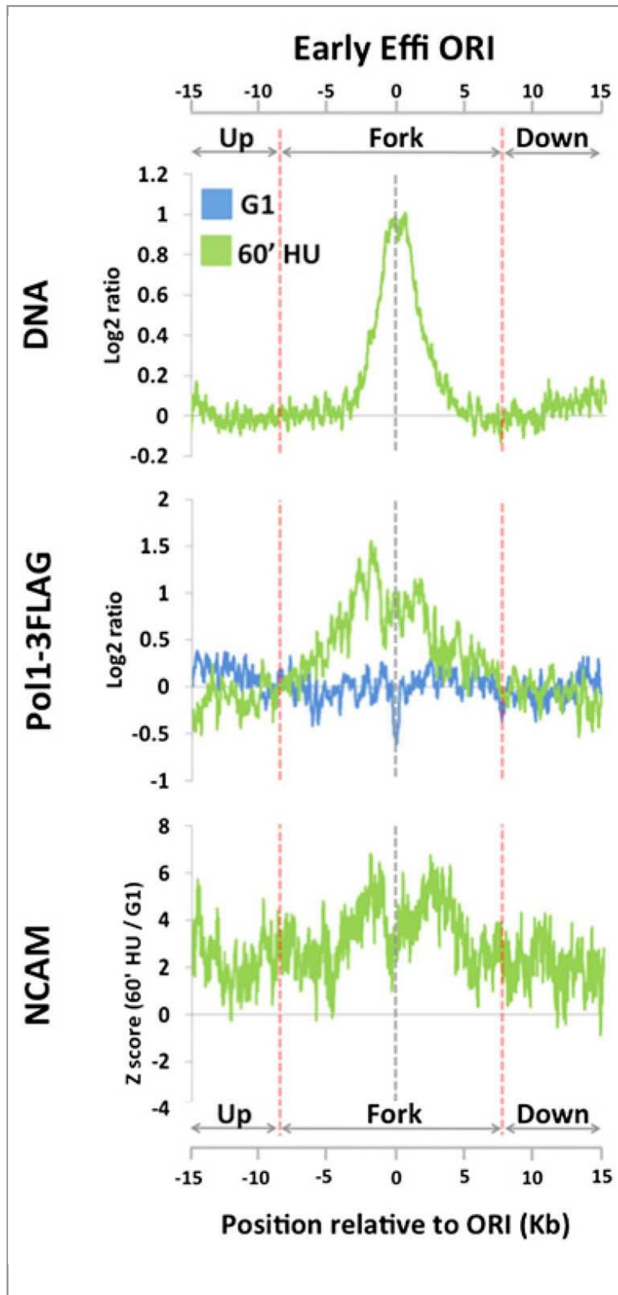

Image obtained from the manuscript

Figure. DNA profiles, DNA polymerase occupancy, and NCAM levels at early ORIs. The green traces represent S-phase samples undergoing DNA replication and blue traces are G1 control. Notice that DNA polymerase I (Pol1-3FLAG) occupancy peaks near ORI position '0' of the fork, similar to the DNA profile. However, NCAM reveals that nucleosome accessibility is greater not only within the fork region, but also upstream and downstream of the fork for several kilobases.