

Fanconi Anemia Proteins are Recruited to Chromatin during Replication and Play a Role in Stabilizing Replication Forks

L.C. Wang¹, J. Gautier¹

¹Columbia University, New York, NY

The activation of the FA pathway and the recruitment of FA proteins to chromatin occur in a DNA replication dependent manner in the presence or absence of DNA damage. This suggests that FA proteins play a role in the detection and/or repair of DNA lesions during replication. However, the molecular requirements for the recruitment of FA proteins to chromatin have not been identified. Furthermore, while a role for FA proteins in the restart of stalled replication forks has been proposed, the exact role of FA proteins has remained elusive.

We are using the *Xenopus* cell free extract system to study the precise timing of FA chromatin binding. We show that FA proteins bind to chromatin after origin assembly and origin firing. MCM-dependent origin unwinding and RPA loading are required for FA chromatin binding whereas DNA polymerase alpha and its enzymatic activities are not required. Immunodepletion of ATR abrogates FANCD2 chromatin binding while inhibition of its kinase activity by caffeine does not. A time course examining the chromatin binding of RPA, ATR, and FA proteins shows that RPA and FA core complex bind concomitantly to chromatin while FANCD2 chromatin binding occurs 30 minutes after initial RPA binding and coincides with ATR binding. These data suggest that RPA binding to single-stranded DNA during replication is sufficient for the chromatin binding of FA core complex proteins, whereas the recruitment of FANCD2 requires an additional role played by ATR, independent of its kinase activity.

We have examined the restart of stalled or collapsed replication forks following the introduction of damaging agents to DNA that has initiated origin firing but not completed elongation. Fork restart is monitored in the presence of caffeine to inhibit checkpoints and of roscovitine, a CDK inhibitor, to prevent firing of new origins. In the absence of FANCL, replication fork restart is reduced following treatment with mitocycin C or camptothecin which cause replication fork collapse.

Translational Applicability: Understanding the specific role of FA proteins at the replication fork may lead to the design of novel therapeutics targeting their function and improve the efficacy of cancer treatments for tumors resistant to crosslinking agents.