

Fanconi Anemia Group D2 Protein Participates in Replication Origin Firing

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Introduction

In the past decade, our laboratory has been greatly interested in studying the Fanconi Anemia (FA) signaling pathway [1-5]. This interest stems from the ability of the FA pathway to maintain genome stability and suppress tumorigenesis. Oncogenic proliferation occurs without sufficient nucleotides to support DNA replication, resulting in replication failure at common fragile sites and leads to the formation of double-strand DNA breaks [6]. The failure in correctly sensing or rescuing stalled replication intermediates is one of underlying causes of several chromosomal instability syndromes such as FA. It is an inherited disorder characterized by congenital abnormalities, bone marrow failure, and cancer proneness [7]. However, much remains unknown regarding how the FA pathway acts under normal conditions and about the manners by which FA proteins contribute a proper progression among individual phases of cell cycle. Moreover, any insight into the FA pathway is important because such insight could lead to understanding of how genomic instability occurs [8]. We expect that the knowledge from understanding this pathway will provide valuable information leading to building up effective tools for cancer prevention as well as therapy.

Successful DNA replication is essential for ongoing cellular life; however, this process poses the threat of DNA damage [9]. Considering the fact that FANCD2 is only monoubiquitinated in the S-phase of each normal cell cycle, our study explored how monoubiquitinated FANCD2 acts during the S-phase of cell cycle [10]. The chromatin binding nature of FANCD2 protein prompted us to reveal that monoubiquitinated FANCD2 has a stronger binding capacity to the replication origins than the non-monoubiquitinated one. Importantly, we observed that the fired origins are reduced in cells deficient in the basal level of monoubiquitinated FANCD2 in comparison with cells carrying a normal level of FANCD2 monoubiquitination. All of these pushed us to go a step further into the mechanisms underlying the functions of monoubiquitinated FANCD2 at replication origins. Through mass spectrometric analysis, we found the interaction between the monoubiquitinated FANCD2 and minichromosome maintenance protein 3 (MCM3). This interaction affects the licensed origin firing of replication at the replication origins. Monoubiquitinated FANCD2 has been studied for a long time for its relationship with DNA damage; but little is known regarding its roles in DNA replication. Our study allow us to first propose a work model regarding how monoubiquitinated FANCD2 performs in DNA replication origin firing and, thus, participates in the control of S-phase progression.

Replication origin firing marks the initiation of DNA replication. This process is regulated through a number of binding-factors, which include the origin recognition complex (ORC) providing a platform for Cdt1 and Cdc6 to recruit MCM2-7 proteins. This complex of helicases unwinds DNA for DNA polymerase to synthesize new DNA [11].

MCM3 was found to be a functional dock for the monoubiquitinated FANCD2 to tether a proper amount of MCM helicase complexes at the origins. This binding relationship appeared to be attributed to the arginine finger region (AA 477-480) of MCM3. And the finding was further supported by the molecular docking using the Z-DOCK web tool [12]. As shown in Figure 1, the predicted structure of human MCM3 (blue), based on the crystalized MCM5 of *Saccharomyces cerevisiae* (PDB: 3JA8.5) using I-TASSER [13-16], adapted well to that of FANCD2 protein (red) (PDB: 3S4W.2). This model also further validated their specific interaction region [MCM3 (magenta) and FANCD2 (cyan)]. Together, our results strongly suggest that this newly recognized role of monoubiquitinated FANCD2 in each S-phase of normally growing cells, will give a post-licensing check on the licensed origins prior to the commencement of firing.

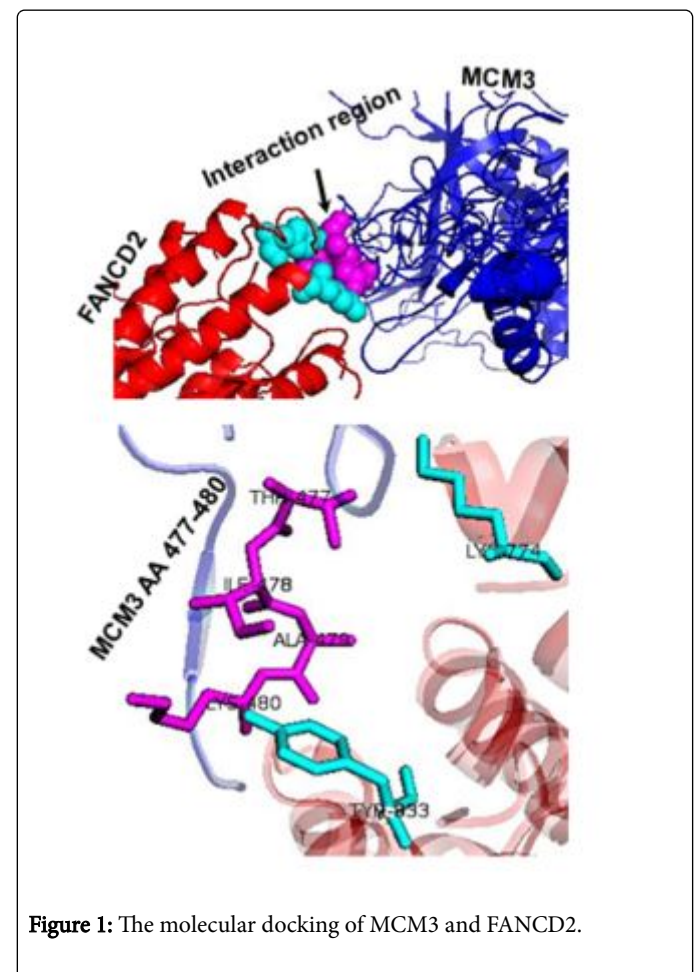
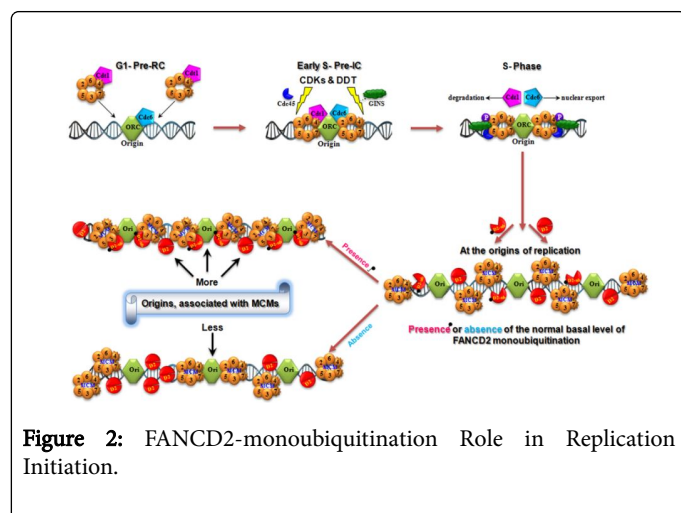


Figure 1: The molecular docking of MCM3 and FANCD2.

Our experiments demonstrate that the basal level of monoubiquitinated FANCD2 is tasked with an active role in replisome surveillance. As shown in Figure 2, the assembling an origin of DNA replication and the normally monoubiquitinated FANCD2 both act in DNA replication initiation. Eukaryotic cells initiate their DNA replication at origins via the interaction between DNA elements (replicators and origins) and protein elements (initiator and other initiation proteins) [17]. Monoubiquitinated FANCD2 in each S-phase interacts with replication origins as well as the member(s) of MCM complex, such as MCM3, to provide “a temporal and spatial check” for securing an adequate amount of licensed-replication origins to fire. Without the normal basal level of FANCD2 monoubiquitination, cells would not have an enough number of licensed origins to initiate a normal replication, thereby overtime rendering genomic instability, aging and cancer, as shown in FA. Our long-term goal is to use the FA pathway as a unique genetic model system to expand our knowledge of tumorigenesis and to use this knowledge to enable preventive and therapeutic approaches for cancer patient care. This has guided us to conduct many studies leading the field of investigation [18,19].



Conclusion

In conclusion, we believe that investigating the FA pathway will allow us to not only understand basic mechanisms underlying DNA repair and response to the cancer treatment but also will be helpful for generating new effective tools in the fight against human cancer. For example, our study would suggest a promising chemotherapeutic approach of targeting specific parts of MCMs to lower down cancer cell progression by screening our Hawaii or other natural products or chemical libraries. The resultant lead compounds would effectively modulate the origin firing of cancer cells and, thus, stop their malignant growth.

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