
Structural insight into the function of full-length human Fanconi Anemia FANCD2/FANCI complex by cryo-electron microscopy

Zuolun Li , Chi-Chao Liang , William Nicholson , Martin Cohn , and Catherine Vénien-Bryan*¹

¹Institut de minéralogie, de physique des matériaux et de cosmochimie (IMPMC) – Museum National d’Histoire Naturelle, Université Pierre et Marie Curie - Paris 6 : UM120, Institut de recherche pour le développement [IRD] : UR206, Centre National de la Recherche Scientifique : UMR7590 – Tour 23 - Barre 22-23 - 4e étage - BC 115 4 place Jussieu 75252 PARIS, France

Résumé

1. INTRODUCTION

At the center of the DNA damage Fanconi Anemia repair pathway is the FANCD2/FANCI complex, which is monoubiquitinated upon DNA damage, and this monoubiquitination is indispensable for activation of the pathway (Ref1). However, neither FANCD2 nor FANCI have any recognizable domains, which results in limited understanding of the molecular functions of the complex. In this study, we have taken a structural approach to better understand the function of the human FANCD2/FANCI complex.

2. Results Experimental methods The native full-length human FANCD2/FANCI complex and the FANCD2/FANCI complex with the C-terminus FANCD2 truncated were purified and subjected to cryo-EM analysis. The activity of the complex was studied *in vivo* and *in vitro* using various assays. Results

Here, we provide the first structural insight into the full-length human FANCD2/FANCI complex utilizing cryo-EM. The complex has a pocket-like main body, a thumb-like structure consisting of the C-terminus of FANCI and a distinctive fork-like tower consisting of the C-terminus of FANCD2 (figure). Deletion of the C-terminus of FANCD2 leads to defective DNA interstrand cross-link repair (ICL repair). FANCD2 lacking the C-terminus cannot be monoubiquitinated, while the *bona fide* monoubiquitination site (Lysine 561) is still present. Furthermore, the association with FANCI and the interaction between FANCD2 and the responsible E3 ligase complex, the FA core complex, remain unaffected in the mutant. The structure of the FANCD2/FANCI complex with the C-terminus FANCD2 truncated was also studied using cryo-EM, this truncated domain is located in the fork-like tower (Figure and ref 2)

3. Conclusion

A new domain of FANCD2 was discovered. The fork-like tower domain in the C-terminus of FANCD2 is required for its complete functions in the ICL repair. Interestingly, several

*Intervenant

disease-causing mutations lie within this region, underscoring the importance of this domain.

Translational Applicability

Our results contribute to uncover the mechanism of how the FANCD2/FANCI complex functions. A better understanding of the molecular mechanism underlying the FA pathway will increase our chances of developing better and more targeted modes of treatment.

References

- 1- Liang CC, Bao Zhan B, Yoshikawa Y, HaasW, Gygi SP, and Cohn MA. **UHRF1 Is a Sensor for DNA Interstrand Crosslinks and Recruits FANCD2 to Initiate the Fanconi Anemia Pathway.** (2015) *Cell reports* **10**, 1947-1956,
- 2- Liang CC, Li Z, Lopez-Martinez D, Nicholson WV, Vénien-Bryan C, Cohn MA **The FANCD2-FANCI complex is recruited to DNA interstrand crosslinks before monoubiquitination of FANCD2.** (2016) *Nat Commun.* **7**:12124.