RGC Ref.:	N_HKU614/17
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The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Cryo-EM Study of Origin Recognition Complexes Bound with Origin DNA

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Yuanliang ZHAI	Ning GAO
Post	Assistant Professor	Professor
Unit / Department / Institution	School of Biological Sciences/HKU	School of Life Sciences/Peking University
Contact Information	zhai@hku.hk	gaon@pku.edu.cn
Co-investigator(s) *(with title and institution)*		Dr. Ningning Li Peking University

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	Jan 01 2018		
Project Completion date	Dec 31 2021		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	Dec 31 2022		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Determine the structures of ORC and ORC-DNA from yeast
2. Explore the role of ScCdc6 in remodelling ScORC for helicase loading
3. Resolve the structures of human ORC complexes

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

To elucidate the mechanism of how yeast ORC (yORC) selects origin DNA to promote MCM loading, we have determined cryo-EM structures of yORC bound to origin DNA. In the yeast ORC-DNA structure, Orc1-5 adopts a closed ring around ACS DNA to grip DNA tightly through both nonspecific contacts with DNA backbone and specific base recognition at ACS site. yOrc6 does not contact ACS DNA but rather is situated in a position to engage with B1 DNA together with Orc2 and Orc5. As a result, DNA is bent away from the central DNA-binding channel of ORC. Our analysis indicates that this special strategic arrangement of origin DNA by ORC may facilitate helicase loading.

We also determine the structure of the apo yORC which exhibits a floppy Orc1-AAA+ and Orc2-WHD. It serves as a DNA entry mechanism in which the gap between Orc1 and Orc2 provides an entry route for origin DNA into the ORC ring channel and the Orc2-WHD functions as a gate controlling DNA access. Interestingly, we found that Cdc6 is inserted transiently into the gap between Orc1 and Orc2 to create contact surfaces for Mcm3 and Mcm7 for the loading of the Cdt1-MCM heptamer. Although the ORC-DNA structure by itself assumes a closed ring

conformation, owing to its flexibility, Orc2-WHD could readily make room to accommodate Cdc6.

Using cryo-EM approach, we further determined the structure of human ORC. The configuration of hORC is drastically different from its yeast counterpart. hORC2-5 resides in a tightly autoinhibited state in which hORC2 largely blocks its DNA binding channel. Upon hORC1 binding, hORC2-5 is transformed into a more dynamic but still-inhibited conformation. Different from yeast, hORC1-5 cannot bind DNA directly, suggesting that further conformational changes in hORC or additional factor(s) are required to promote its loading onto origin DNA. This information also highlights the differences of the regulation of ORC activities in yeast and human.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

The fine details of the structures produced in this study will serve as a rich source of information for designing as well as interpreting biochemical studies aiming at dissecting the mechanistic functions of the ORC in its many biological roles. In particular, it will provide a framework for future study of origin selection and helicase loading.

7. The Layman's Summary

(describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

All living creatures, from simple unicellular yeast to complex multicellular human being, propagate through cell divisions. Each division requires the exact replication of the genome DNA, which is the blueprint of the identity of every organism. Over-replication may lead to cancers and under-replication may lead to developmental defects such as Meier-Gorlin syndrome. DNA replication is initiated at replication origins by ORC and other protein complexes assembled at these sites.

Structure informs function. The high-resolution structures of the ORC isolated from yeast and human help us to better understand the roles of ORC in regulating our DNA replication. Our studies lay a solid foundation to identify pairs of interactions, that are critical for origin recognition and helicase loading, with the potential as targets for anticancer drug screening and design.

<u>Part C: Research Output</u>

8. Peer-reviewed journal publication(s) arising <u>directly</u> from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications	Author(s)	Title and	Submitted to	Attached	Acknowledge	Accessible

Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	RGC *(indicate the year ending of the relevant progress report)*	to this report *(Yes or No)*	d the support of this Joint Research Scheme *(Yes or No)*	from the institutional repository *(Yes or No)*
2020				Jiaxuan Cheng, **Ningning Li*,** Xiaohan Wang, Jiazhi Hu, **Yuanliang Zhai**, **Ning Gao***	Structural insight into the assembly and conformational activation of autoinhibited human origin recognition complex **Cell Discovery** (6:88)		Yes	Yes	Yes
2018				**Ningning li,** Wai Hei lam, **Yuanliang Zhai***, Jiaxuan cheng, erchao cheng, Yongqian Zhao, **Ning Gao***, Bik-Kwoon tye*	Structure of the origin recognition complex bound to DNA replication origin **Nature** (559, 217-222)		Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered

(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*

June/2018/Suzhou China	Structure of the origin recognition complex bound to the essential elements of ARS DNA	DNA Metabolism, Genomic Stability & Human Disease—Cold spring harbour meeting Asia		Yes	Yes	No
June/2019/Beijing China	Cryo-EM structures of the origin recognition complex bound to the essential elements of ARS DNA	The Sixth National Symposium of Cryo-EM and Structural Biology		Yes	Yes	No
Sep/2019/New York/US	Cryo-EM structures of the origin recognition complex bound to the essential elements of ARS DNA	EUKARYOTIC DNA REPLICATION & GENOME MAINTENANCE		Yes	Yes	No
Dec/2019/Singapore	From structure to function: Cryo-EM structures of the origin recognition complex	16th Conference of the Asian Crystallographic Association		Yes	Yes	No

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

NA

12. Statistics on Research Outputs *(Please ensure the summary statistics below are consistent with the information presented in other parts of this report.)*

	Peer-reviewed journal publications	Conference papers	Scholarly books, monographs and chapters	Patents awarded	Other research outputs (Please specify)
No. of outputs arising directly from this research project [or conference]	2				