CRF 8G (Revised Sep 15)

RGC Reference HKBU5/CRF/11G
please insert ref. above

The Research Grants Council of Hong Kong
Collaborative Research Fund Group Research Projects
Completion Report

(*for completed projects only*)

Part A: The Project and Investigator(s)

1. Project Title

Defining the Regulatory Pathways Coupling Cell Division Timing and Cell Fate Differentiation During *C. elegans* Embryogenesis Using Automated Lineaging

2. Investigator(s) and Academic Department/Units Involved *(please highlight approved changes in the composition of the project team and quote the date when RGC granted approval of such changes)*

Research Team	Name/Post	Unit/Department/Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator	Zhongying Zhao/Associate professor	Biology/Hong Kong Baptist University	10
Co-Principal investigator(s)	Hong Yan/Chair professor	Electronic Engineering /City University of Hong Kong	2
Co-Principal investigator(s)	King Chow/Professor	Division of Life Science/Hong Kong University of Science and Technology	2
Co-Principal investigator(s)	Yiji Xia/Professor	Biology/Hong Kong Baptist University	1
Collaborators/ Others			

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted)*
Project Start Date	May 1st 2012	NA	
Project Completion Date	April 30th 2015	April 30th, 2016	January 12nd 2015
Duration (*in month*)	36	48	
Deadline for Submission of Completion Report	October 17th, 2015	October 16th, 2015	

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Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. We will deplete activities for approximately 1100 genes prioritized based on their relevance to embryogenesis and human biology by RNAi through microinjection.
2. We will automatically measure the defects in cell division timing and cell fate determination for the F1 embryos produced in the Objective 1 for every cell using the tools our group has recently developed.
3. We will develop statistical methods for systematic quantification and comparison of changes in cell division timings associated with homeotic cell fate transformation, thus infer the regulatory relationship by phenotype clustering.
4. We will extrapolate one validated pathway in other organism by studying the *in vivo* roles of the orthologs of the genes within the pathway in mouse.

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5.2 Revised objectives

Date of approval from the RGC: February 13rd 2012

Reasons for the change: Substantial budget cut: 6.664 million HKD originally proposed; revised budget: 5 million HKD

1. We will deplete activities for approximately 900 genes prioritized based on their relevance to embryogenesis and human biology by RNAi through microinjection.
2. We will automatically measure the defects in cell division timing and cell fate determination for the F1 embryos produced in the Objective 1 for every cell using the tools our group has recently developed.
3. We will develop statistical methods for systematic quantification and comparison of changes in cell division timings associated with homeotic cell fate transformation, thus infer the regulatory relationship by phenotype clustering.

6. Research Outcome

6.1 Major findings and research outcome

(maximum 1 page; please make reference to Part C where necessary)

- A high-content screen for *C. elegans* genes whose depletion affects asynchrony in cell division demonstrates that fate determinants are not only essential for establishing fate asymmetry, but also important in controlling division asynchrony regardless of cellular context (see publication in Mol Syst Biol in Part C).
- The screen identified many genes with unknown functions that are involved in regulating cell division asynchrony (see publication in Mol Syst Biol in Part C).
- The screen combined with biochemical study identified GAD-1 as a novel member of splicesome that is required for intestine precursor-specific cell division timing and gastrulation (under review, PLoS Genetics, see separate attachment)
- Perturbation of cell fate determinants involved in the regulation of division pace frequently leads to a defective migration of the same cell (see publication in Mol Syst Biol in Part C).
- Tissue-specific increase in cell division timing in intestine precursors is due to robust initiation of zygotic expression triggered by Wnt signaling event at a four-cell staged embryo (See publication in JBC, 2016).
- There is collaborative regulation of development but independent control of metabolism by two epidermis-specific transcription factors, NHR-25 and ELT-1, in *C. elegans* (See publication in JBC, 2013).
- The quantitative data with cellular resolution (available in the "Phenics" Database http://phenics.icts.hkbu.edu.hk/) constitute an invaluable resource for inferring cell-specific gene

pathways controlling spatiotemporal coordination during fate specification or tissue growth (see publication in Mol Syst Biol in Part C).

- Multiple novel algorithms on gene network inference have been developed (See publications in Bioinformatics, 2014; Nucleic Acids Res. 2014; Mol Biosyst. 2016)
- A novel algorithm on image segmentation was produced. (See publications in BMC Bioinformatics, 2014; IEEE Journal of Selected Topics in Signal Processing, 2016, on which a US patent was filed (see separate attachment))
- A novel algorithm on inference of signal/noise ratio of gene expression was developed using Bayesian detection (See publication in Annals of Applied Statistics).

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

Thanks to the exceptional temporal and spatial resolution of the data produced in the project, the output of this project has laid down a solid basis for further studying the interaction between cell cycle asynchrony and cell fate specification, as well as the precise roles of cell signaling in defining both temporal and fate asymmetries with cellular resolution at one-minute interval during proliferative stage of metazoan development. Specifically, alignment of division asymmetry with linear expression of signaling molecules such as those from Notch or Wnt signaling pathways will be very useful in delineating the signaling event responsible for the individual asynchrony. Correlating regulation between asynchrony and fate asymmetry of the same cell will provide insights into how cell type-specific division durations are acquired during embryogenesis.

6.3 Research collaboration achieved *(please give details on the achievement and its relevant impact)*

International collaborations with co-publications:

1. Prof. Bob Waterston at University of Washington, Seattle, WA, USA; Co-authored paper in Genome Research, 2012.
2. Prof. John Stamatoyannopoulos at University of Washington, Seattle, WA, USA; Co-authored paper in JBC, 2013.
3. Dr. Peter Sarkies at Imperial College London, UK on characterization of small RNAs in nematode hybrids; Co-authored paper in Genome Research, 2016.
4. Dr. Zhihong Zhang at Illumina, USA, on male sterility and nematode genome project; Co-authored paper in Genome Research, 2016.

Local collaborations with co-publications:

5. Prof. Hong Yan, Department of Computer Engineering, City University of Hong, Co-authored multiples paper, including one Molecular Systems Biology, BMC Bioinformatics, Molecular Biosystems. He is the Co-PI of this CRF grant.
6. Prof. King Chow, Division of Life Sciences, University of Science and Technology, Hong Kong. Co-authored paper in Molecular Systems Biology. He is the Co-PI of this CRF grant.
7. Prof. Yiji Xia, Department of Biology, Hong Kong Baptist University, Hong Kong. Co-authored paper in Molecular Systems Biology. He is the Co-PI of this CRF grant.
8. Dr. Junwen Wang, Department of Biochemistry, the University of Hong Kong. Co-authored paper in Bioinformatics.
9. Dr. Xiaodan Fan at Department of Statistics, Chinese University of Hong Kong on statistical analysis of gene expression with cellular resolution with one-minute interval; Co-authored paper in Annals of Applied Statistics.

Collaborations in mainland China:

10. Prof. Long Miao, at Institute of Biophysics, Chinese Academy of Science, Beijing, China. Co-authored paper in Genome Research, 2016.
11. Dr. Rene Huang at Tsinghu University, China on isolation and identification of new *Caenorhabditis* species. Co-authored papers in PLoS One, Invertebrate Systematics.
12. Dr. Mengqiu Dong, National Institute of Biological Sciences, Beijing, China. Collaborating on the characterization of GAD-1 biochemical function. Co-authored manuscript under review in PLoS Genetics.

7. The Layman's Summary

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

Propagation of a cellular organism from one generation to another requires both cell division and cell growth. Cell divisions of single-celled organisms are independent of each other while those of a multicellular organism require tight coordination among one another in order to form different cell types. Failure in the coordination frequently leads to abnormal cell death or tumorous growth. How the cell division paces are coordinated to ensure formation of proper cell types during animal development remains poorly understood. By a combination of biological and computing science, our previous group developed multiple tools allowing automatic tracing of cell division histories during *C. elegans* embryogenesis. We applied these tools to identify genes that couple cell division paces with cell fate differentiation. We find that genes used for specifying cell fate are also required for coordinating division paces of cells regardless of their fate during embryogenesis, in which cells are undergoing rapid divisions that are concurrent with cell fate specification. Coordination of cell paces appears to be essential for proper cell migration during metazoan development. The knowledge obtained provides mechanistic insight into how cell division paces are tuned *in vivo* to accommodate tissue formation and cell fate specification at cellular resolution during animal development, which sheds light on how a cancer cell is originated in the first place.

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Part C: Research Output

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

(Please attach a copy of the 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) *(denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance (For paper accepted but not yet published)	Under Review	Under Preparation (optional)						
2016				Li R, Ren X, Bi Y, Ho VW, Hsieh CL, Young A, Zhang Z, Lin T, Zhao Y, Miao L, Sarkies P*, Zhao Z.*	Specific Downregulation of Spermatogenesis Genes Targeted by 22G RNAs in Hybrid Sterile Males Associated with an X-Chromosome Introgression. ***Genome Research***.	2016	Yes	Yes	Yes
2016				Wong MK, Guan D, Ng KH, Ho VW, An X, Li R, Ren X, Zhao Z.*	Timing of Tissue-specific Cell Division Requires a Differential Onset of Zygotic Transcription during Metazoan Embryogenesis. ***J Biol Chem***.	2016	Yes	Yes	Yes

2016				Chen Long, Zhongying Zhao, Hong Yan *	A Probabilistic Relaxation Labeling (PRL) Based Method for *C. elegans* Cell Tracking in Microscopic Image Sequences. ***IEEE Journal of Selected Topics in Signal Processing***	2016	Yes	Yes	Yes
2016				Huang XT, Zhu Y, Chan LL, Zhao Z, Yan H*	An integrative *C. elegans* protein-protein interaction network with reliability assessment based on a probabilistic graphical model. ***Mol Biosyst***	2016	Yes	Yes	Yes
2015				WS Ho, MKWong, XM An, DG Guan, JF Shao, HC Ng, XL Ren, K He, J Liao, Y Ang, L Chen, X Huang, B Yan, Y Xia, LH Chan, KL Chow, H Yan and Z Zhao*	Systems-level quantification of division timing reveals a common genetic architecture between asynchrony and fate asymmetry. ***Molecular***	2016	Yes	Yes	Yes

					Systems Biology				
2015				Yu Bi, Xiaoliang Ren, Cheung Yan, Jiaofang Shao, Dongying Xie and Zhongying Zhao*	A Genome-Wide Hybrid Incompatibility Landscape between *Caenorhabditis briggsae* and *C. nigoni*. ***PLoS Genet***.	2016	Yes	Yes	Yes
2015				Runsheng Li, Chia-Ling Hsieh, Amanda Young, Zhihong Zhang, Xiaoliang Ren and Zhongying Zhao*	Illumina Synthetic Long Read Sequencing Allows Recovery of Missing Sequences even in the "Finished" *C. elegans* Genome. ***Scientific Reports***	2016	Yes	Yes	Yes
2015				Jie Hu*, Zhongying Zhao, Hari Krishna Yalamanchili, Junwen Wang, Kenny Ye, Xiaodan Fan*	Bayesian detection of embryonic gene expression onset in *C. elegans*. ***Annals of Applied Statistics*** .	2016	Yes	Yes	No
2015				Huang RE*, Ye W, Ren X, and **Z Zhao***	Morphological and Molecular Characterization of Phasmarhabditis huizhouensis sp. nov. (Nematoda	2016	Yes	Yes	Yes

					: Rhabditidae), a New Rhabditid Nematode from South China. ***PLoS One***				
2014				Huang RE, Ren X, Qiu Y, Zhao Z*	Description of Caenorhabditis sinica sp. n. (Nematoda: Rhabditidae), a nematode species used in comparative biology for C. elegans. ***PLoS On***e	2016	Yes	Yes	Yes
2014				Guan D, Shao J, Zhao Z , Wang P, Qin J, Deng Y, Boheler KR, Wang J, Yan B*	PTHGRN: unraveling post-translational hierarchical gene regulatory networks using PPI, ChIP-seq and gene expression data. ***Nucleic Acids Res***	2016	Yes	Yes	Yes
2014				He K, Zhou T, Shao J, Ren X, Zhao Z, Liu D*	Dynamic regulation of genetic pathways and targets during aging in *Caenorhabditis elegans*. ***Aging (Albany***	2016	Yes	Yes	Yes

					NY)				
2014				Daogang Guan, Jiaofang Shao, Youping Deng, **Zhongying Zhao**, Yan Liang, Junwen Wang, Bin Yan*	CMGRN: a web server for constructing multi-level gene regulatory networks using ChIP-seq and gene expression data. ***Bioinformatics***	2016	Yes	Yes	Yes
2013				Hari Krishna Yalamanchili, Bin Yan, **Zhongying Zhao**, Francis YL Chin, Mulin Jun Li, Jing Qin and Junwen Wang*	DDGni: dynamic delay gene-network inference from high-temporal data using gapped local alignment. ***Bioinformatics***	2016	Yes	Yes	Yes
2013				Long Chen*, Leanne Lai Hang Chan, Zhongying Zhao and Hong Yan	A novel cell nuclei segmentation method for 3D C. elegans embryonic time-lapse images. ***BMC Bioinformatics***	2016	Yes	Yes	Yes
		√		Xiaomeng An, Ming-Kin Wong, Wen-Jun Li, Vincy Wing Sze Ho,	Highly Conserved Protein GAD-1 Regulates Gastrulation and	2016	Yes	Yes	No

				Daogang Guan, Runsheng Li, Derek Hoi-Hang Ho, Xiaoliang Ren, Hailei Zhang, Yiji Xia, Roger H. F. Wong, Meng-Qiu Dong, Zhongying Zhao*	Tissue-Specific Cell Division Timing by Working as a Novel Component of Spliceosome. ***PLoS Genetics***				
2013				Shao J, He K, Wang H, Ho WS, Ren X, An X, Wong MK, Yan B, Xie D, Stamatoyannopoulos J*,Zhao Z*	Collaborative Regulation of Development but Independent Control of Metabolism by Two Epidermis Specific Transcription Factors in *C. elegans*. ***J Biol Chem***	2014	Yes	Yes	Yes
2013				Huang X*, Chen L, Chim H, Chan LL, Zhao Z, Yan H	Boolean genetic network model for the control of C. elegans early embryonic cell cycles. ***Biomed Eng Online***	2014	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 conference abstract)*

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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 RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
July, 2016, Beijing, China	Timing of Tissue-specific Cell Division Requires a Differential Onset of Zygotic Transcription during Metazoan Embryogenesis (Oral presentation)	8th Asia Pacific C. elegans Development, Cell Biology and Gene Expression Meeting	2016	Yes	Yes	No
June, 2015	Illumina Synthetic Long Read Sequencing Allows Recovery of Missing Sequences even in the "Finished" C. elegans Genome (Poster)	2015 International C. elegans meeting	2016	Yes	Yes	No
June, 2014	Defining regulatory pathways coupling cell division timing and cell fate differentiation in *C. elegans* by automated lineaging (Oral presentation)	8th Asia Pacific C. elegans Development, Cell Biology and Gene Expression Meeting	2016	Yes	Yes	No
06/2013	Defining regulatory pathways coupling cell division timing and cell fate differentiation in *C. elegans* by automated lineaging (Oral presentation)	19th International C. elegans Meeting	2014	Yes	Yes	No
06/2013	A Genome-Wide Hybrid Incompatibility Landscape between *Caenorhabditis briggsae* and *C. nigoni*.	19th International C. elegans Meeting	2014	Yes	Yes	No
06/2013	Independent Regulation of Metabolism but Coordinated Control of Tissue Development by Epidermis Specific Proteins in Caenorhabditis elegans (poster)	19th International C. elegans Meeting	2014	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

Vincy Wing Sze Ho	PhD	September 1st, 2012	August 31st, 2016
Xiaoliang	PhD	September 1st, 2013	August 31st, 2016
Long Chen	PhD	July 1st, 2013	June 30st, 2016
Ming Kin Wong	Mphil	September 15st, 2013	September 14st , 2015
Yu Bi	Mphil	September 1st, 2012	August 31st, 2014

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

Two publications, one in Molecular Systems Biology (5 yr Impact Factor of 12.34) and other in PLoS Genetics (5 yr Impact Factor of 7.5), were highlighted as one of the significant research advances in animal genetics in China in 2015 (see separate attachment). The paper in PLoS Genetics was also selected by the Faculty of 1000.

A provisional US patent was submitted based on an algorithm developed for cell image segmentation (see separate attachment).

Project Coordinator

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Website dedicated to the CRF project: http://phenics.icts.hkbu.edu.hk/index.php?r=site/index

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