25th TMIMS

International Symposium on Cells and Chromosomes

date

2023.10.16 | mon. | 9:30-17:00

venue

TMIMS Auditorium

speakers

Charlie M. Boone Toronto University, Canada; RIKEN, Japan
Marco Foiani IFOM, Italy
Daochun Kong Peking University, China
Frank Uhlmann Francis Crick Institute, UK
Li Qing Peking University, China
Dirk Remus Memorial Sloan Kettering Cancer Center, USA
Tomohiro Iguchi TMIMS, Japan
Hideya Kawaji TMIMS, Japan
Hiroyuki Sasanuma TMIMS, Japan

organized by

Hisao Masai TMIMS, Tokyo, Japan

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TMiMS

Preface

This meeting marks 25th International Symposium at Tokyo Metropolitan Institute of Medical Science (TMiMS). The theme of this symposium is ***Cells and Chromosomes.***

In this symposium, we have six distinguished invited speakers from abroad and three speakers from TMiMS, who will tell us the forefront of cell and molecular biology, genomics, genetics, and biochemistry on various chromosome transactions including histone regulation, replication, recombination, transcription and cellular genetic networks. We will also discuss how understanding of the basic mechanisms of chromosome regulation will lead to conceptual transition toward understanding of diseases and development of novel diagnosis and therapies.

We hope that this symposium will stir the interest of scientists working on various fields of medical sciences and enhance productive collaboration between those with different expertise to achieve our common goal of improving health and welfare of human being.

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Hisao Masai

On behalf of the organizing committee

Program

9:00-9:30 Registration

9:20-9:30 Introduction - Hisao Masai (The Director General of TMIMS)

9:30-10:35 Session 1, Chair: Naoko Yoshizawa (TMIMS)

9:30-10:10 Qing Li (Peking University)
"Navigating traffic at replication forks: Replisome-histone chaperone collaboration in guiding the histone partitioning"

10:10-10:35 Tomohiro Iguchi (TMIMS)
"Association of mammalian Rif1 with nuclear membrane is essential for genome-wide replication timing regulation"

10:35-10:55 Coffee Break

10:55-12:05 Session 2, Chair: Yasuko Ono (TMIMS)

10:55-11:25 Hideya Kawaji (TMIMS)
"Unraveling the genomic code in gene regulation"

11:25-12:05 Charlie Boone (University of Toronto)
"Mapping Genetic Networks in Yeast and Human Cells"

12:05-13:15 Lunch

13:15-15:05 Session 3, Chair: Hisao Masai(TMIMS)

13:15-13:45 Hiroyuki Sasanuma (TMIMS)
"BRCA1 promotes repair of estrogen-induced and topoisomerase II-dependent DNA double-strand breaks"

13:45-14:25 Marco Foiani (The FIRC Institute of Molecular Oncology)
"Confined cell migration is genotoxic and oncogenic"

14:25-15:05 Frank Uhlmann (The Francis Crick Institute)
"To know your sister: The establishment of sister chromatid cohesion during DNA replication"

15:05-15:25 Coffee Break

15:25-16:45 Session 4, Chair: Li Qing (Peking University)

15:25-16:05 Dirk Remus (Memorial Sloan Kettering Cancer Center)
"Biochemical analysis of programmed and unscheduled eukaryotic replication fork stall events"

16:05-16:45 Daochun Kong (Peking University)
"Cellular Regulation and Stability of DNA Replication Forks in Eukaryotes"

16:45-17:00 Conclusions: Frank Uhlmann (The Francis Crick Institute)

Qing Li

Peking University, Beijing, China

NAME Qing Li, PhD (李晴)
POSITION Professor
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EDUCATION

2001-2006	Ph.D. Biochemistry and Molecular Biology Peking University, China
1997-2001	B.S. Biochemistry and Molecular Biology Peking University, China

PROFESSIONAL APPOINTMENTS

2019-present	Professor School of Life Sciences, Peking University
2017-2018	Associate Professor School of Life Sciences, Peking University
2012-2017	Assistant Professor School of Life Sciences, Peking University
2012-present	Principle Investigator Peking-Tsinghua Center for Life Sciences
2006-2011	Postdoc Mayo Clinic, Rochester, MN

HONORS AND AWARDS

2022	The Excellence in Mentoring Award, Peking University
2021	Teaching Excellence Award (Undergraduate), Peking University
2018	The Beijing Outstanding Young Scientist Program, Beijing

	Municipal Education Commission
2017	The Distinguished Young Scientist Award, Ministry of Education, China
2017	National Natural Science Funds for Distinguished Young Scholar

SELECTED PUBLICATIONS

1. Wang XZ et al., (2023) The N-terminus of Spt16 anchors FACT to MCM2-7 for parental histone recycling. *Nucleic Acids Research* (Accepted)
2. Leng H et al., (2021) FACT interacts with Set3 HDAC and fine-tunes GAL1 transcription in response to environmental stimulation. *Nucleic Acids Research*, 49:5502-5519
3. Xu ZY et al., (2021) Measuring Genome-Wide Nascent Nucleosome Assembly Using Replication-intermediate nucleosome mapping (ReIN-Map). *Methods Molecular Biology* (Book) 2196:117-141.
4. Li SQ et al., (2018) Rtt105 functions as a chaperone for replication protein A to preserve genome stability. *The EMBO Journal* e99154.
5. Liu SF et al., (2017). RPA binds histone H3-H4 and functions in DNA replication-coupled nucleosome assembly. *Science* 355, 415-420.
6. Yang et al., and Li, Q.* (2016). The Histone Chaperone FACT Contributes to DNA Replication-Coupled Nucleosome Assembly. *Cell Reports* 14, 1128-1141.

Navigating traffic at replication forks: Replisome-histone chaperone collaboration in guiding the histone partitioning

Qing Li
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The replication of eukaryotic chromosomes is a crucial process for epigenetic inheritance, requiring both DNA duplication and chromatin reassembly. One important aspect of this process is the coordination of histone traffic at replication forks, involving two pools of histone H3-H4 tetramers: recycled parental histones and newly synthesized histones. These histones are then distributed to the leading and lagging strands of the replication fork. However, the precise mechanisms underlying the coordination of histone traffic at replication forks remain to be fully determined. Accumulating evidence suggests the involvement of two critical groups of factors in histone traffic at replication forks. The first group consists of histone chaperones, which play a role in the assembly and disassembly of nucleosomes coupled with replication. The second group comprises several replisome components that can bind histones and act as co-chaperones. In this meeting, I will present our recent progress in understanding how histone chaperones collaborate with replisome components to guide histone traffic at replication forks. Specifically, I will discuss how the single-stranded binding protein Replication Protein A (RPA) acts as a central ”hub” for organizing the nucleosome assembly of both parental and newly synthesized H3-H4. Additionally, I will highlight the role of the histone chaperone FACT in anchoring the replisome to coordinate the recycling and transfer of parental histones at replication forks. By considering the relative positions of these replisome components at the replication fork and their interactions with histone chaperones, we propose a model called the ”Departure-Hub-Terminal” model. This model aims to explain the functions of these proteins, as well as others, in orchestrating parental

histone recycling and the deposition of new H3-H4 on the leading and lagging strands. We believe that the efficient collaboration between histone chaperones and the replisome governs the efficiency, balance, and symmetry of histone traffic during the chromatin replication process. This collaboration ultimately enables the inheritance of epigenetic information.

Tomohiro Iguchi

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EDUCATION & DEGREE

2009 B.S., Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science
2011 M.S., Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science
2018 PhD., Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science

EMPLOYMENT

2014 Research assistant, Laboratory of Self Defense Gene Regulation, Tokyo Metropolitan Institute of Medical Science
2016 Research assistant, Laboratory of BioMembrane, Tokyo Metropolitan Institute of Medical Science
2018 Postdoctoral Researcher, Genome Dynamics Project, Tokyo Metropolitan Institute of Medical Science
2019~ Researcher, Genome Dynamics Project, Tokyo Metropolitan Institute of Medical Science

PUBLICATIONS

Iguchi, T., et al. *J Immunol* 195, 982-93 (2015)
Ohtsuka H., et al. *PLoS One* 12, e0169609 (2017)
Iguchi, T., et al. *Bochum Biophys Res Commun* 501, 570-575 (2018)

Komatsuya, K., et al. *J Neurochem* 163, 375-390 (2022)
Hori, K., et al. *Genes Cells* in press (2023)

Association of mammalian Rif1 with nuclear membrane is essential for genome-wide replication timing regulation

Tomohiro Iguchi[1], Sayuri Ito[1], Naoko Kakusho[1], Rino Fukatsu[1], Kenji Moriyama[1], Asako Sawano[2], Asami Oji[3], Mikihiro Shibata[4], Atsushi Miyawaki[2], Ichiro Hiratani[3], Hiroyuki Sasanuma[1], Hisao Masai[1]

[1] Tokyo Metropolitan Institute of Medical Science, Genome Dynamics

[2] RIKEN CBS, Team for Cell Function Dynamics

[3] RIKEN BDR, Laboratory for Developmental Epigenetics

[4] Kanazawa University, NanoLSI

Rif1, an evolutionally conserved nuclear factor, regulates genome-wide replication timing by its ability to recruit phosphatase and by its potential to generate higher-order chromatin structures near nuclear periphery. Mammalian Rif1 is composed of N-terminal HEAT/Armadillo repeats, central long IDP and a C-terminal unknown domain. We previously proposed that fission yeast Rif1 regulates genome-wide DNA replication timing by generating the chromatin architecture though its ability to bind to G-quadruplex (G4) and form oligomers. A portion of mammalian Rif1 is localized near nuclear periphery and is biochemically fractionated into detergent-resistant membrane fractions. We show that Rif1 associates with endomembrane and this association requires small stretches of amino acids near the C-terminus of Rif1. Point mutations in the C-terminal segment of Rif1 lead to its dissociation from the nuclear periphery, loss of mid-S replication foci pattern, perturbation of multimer formation and dramatically altered replication timing throughout the genome with subsets of replication timing domains being converted from late to early. Membrane association of Rif1 may be mediated by two distinct mechanisms; direct lipid binding through the C-terminal amphipathic coiled-coil structure and lipid modification through palmitoylation. We will discuss mechanisms of nuclear membrane association of Rif1 and its cell cycle regulation, and how it may contribute to chromatin structures and spatio-temporal regulation of DNA replication.

Hideya Kawaji

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EDUCATION & DEGREE

1997 B.S. in School of engineering science, Osaka University
1999 M.S. in School of engineering science, Osaka University
2003 Ph.D. in School of engineering science, Osaka University

EMPLOYMENT

1999 NTT Software Corp.
2007 Research Scientist, RIKEN Omics Science Center
2009 Visiting Associate Professor, Graduate School of Nanobioscience, Yokohama City University
2011 Unit Leader, Small RNA Analysis Collaboration Unit, RIKEN Omics Science Center
2013 Coordinator, RIKEN Preventive Medicine and Diagnosis Innovation Program
2014 Unit Leader, Preventive Medicine and Applied Genomics unit, RIKEN Advanced Center for Computing and Communication
2018 Unit Leader, Preventive Medicine and Applied Genomics unit, RIKEN Center for Integrative Medical Sciences
2019 Investigator, Tokyo Metropolitan Institute of Medical Science
2020- Associate Center Director, Research Center for Genome & Medical Sciences, Tokyo Metropolitan Institute of Medical Science

EDITORIAL ACTIVITIES
Editorial Board member, Scientific data (Springer Nature) (2014˜)

AWARDS
2010　RIKEN Research Encouragement Award
2013　RIKEN Omics Science Center OSCAWARD
2014　JSBi Research Encouragement Award

SELECTED PUBLICATIONS
Forrest, A. R. R., et al. *Nature* 507, 462–470 (2014)
Kawaji, H., et al. *Genome Res.* 24, 708–717 (2014)
Hirabayashi, S., et al. *Nat. Genet.* 51, 1369–1379 (2019)
Ito, Y., et al. *Sci. Rep.* 11, 9355 (2021)
Abugessaisa, I., et al. *Nucleic Acids Res.* 49, D892–D898 (2021)

Unraveling the genomic code in gene regulation

Hideya Kawaji
Research Center for Genome & Medical Sciences, Tokyo Metropolitan Institute of Medical Science

Cis-regulatory element (CRE) is a genomic region to regulate transcription. This includes promoters, which are located at proximal to transcription initiation sites, and enhancers, which are located at distal to these sites. The genomic code that forms CREs specifies the gene regulatory program, and it is crucial to accurately map their location within the genome and identify their targets in order to understand the impact of genetic or epigenetic alterations on our phenotypes. Genome-wide profiling of epigenomic and transcriptomic signatures associated with CREs, such as opened chromatin combined with a set of histone modifications and patterns of transcription initiation, have successfully identified candidate CREs. However, the complete picture of CREs remains elusive due to the fact that CREs are specific to cellular states and genomic signatures often co-occur with other functional elements.

We approached to the gene regulatory program encoded in the human genome from multiple aspects. We have developed a novel computational method to identify CREs based on a single assay of genome-wide profiling of transcription starting site with CAGE (Cap Analysis of Gene Expression). This method achieved compatible sensitivity to a previous method that relied on multiple epigenetic assays. Application of this method led us to identify overlooked drug-inducible enhancers that may impact on individual differences in drug metabolism. We further found enrichment of enhancers around the transcription termination sites. Through the use of two independent design of massively parallel reporter assays (MPRA) including STARR-seq, we found that genomic regions can serve both transcriptional and post-transcriptional regulations. Our results emphasizes that the complexity of genomic code regulating gene expressions.

Charles M. Boone

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Biographical Information

1. Degrees:

09/1978-09/1982 B.Sc. Queens University, Chem. & Math
09/1984-09/1989 Ph.D. McGill University, Biology

2. Employment:

07/2000 Associate Professor, Banting and Best Dept of Medical Research, Dept. of Molecular Genetics, University of Toronto
07/2001 Associate Professor, tenure, Banting and Best Dept of Medical Research, Dept. of Molecular Genetics, University of Toronto
07/2003 Professor, The Donnelly Centre, Dept. of Molecular Genetics, University of Toronto
03/2019-09/2019 Acting Director, The Donnelly Centre, University of Toronto
07/2020-08/2021 Interim Director, The Donnelly Centre, University of Toronto

3. Honors and Awards

- Member, American Academy of Arts & Sciences, 2022
- Foreign Member, Royal Swedish Academy of Sciences, 2021
- Banting & Best Distinguished Scholar, 2020-present, University of Toronto, Faculty of Medicine
- Genetics Society of America (GSA) Edward Novitski Prize, Creativity in Genetics, 2014
- The Emil Christian Hansen Award for Microbiology, 2013, The Carlsburg Foundation, Copenhagen (with Brenda Andrews).

- International Research Scholar, Howard Hughes Medical Institute, 2012-2017.
- Fellow, American Academy of Microbiology, 2012- present.
- Honorary Doctorate Degree, University of Gothenburg 2011.
- Tanenbaum Chair, Molecular Medicine, University of Toronto, 2011-2021.
- Fellow of the American Association for the Advancement of Science, 2011-present.
- Canada Research Chair (Tier 1), Proteomics, Bioinformatics and Functional Genomics, 2007-2021.
- Ira Herskowitz Award, Yeast Genetics and Molecular Biology Meeting, 2006.

1. Kuzmin E†, VanderSluis B†, Wang W, Tan G, Deshpande R, Chen Y, Usaj M, Balint A, Mattiazzi Usaj M, van Leeuwen J, Koch EN, Pons C, Dagilis AJ, Pryszlak M, Want Z, Xu K, Heydari H, San Luis B-J, Shuteriqi E, Zhu H, van Dyk N, Sharifpoor S, Costanzo M, Bolnick D, Brown GW, Andrews BJ*, **Boone C***, Myers CL* (2018). Systematic analysis of complex genetic interactions. *Science*. 360(6386). pii: eaao1729. doi: 10.1126/science.aao1729.
2. Piotrowski JS†, Li SC†, Deshpande R†, Simpkins SW†, Nelson J, Yashiroda Y, Barber J, Safizadeh H, Wilson E, Okada H, Gebre AA, Kubo K, Torres N, Leblanc MB, Andrusiak K, Okamoto R, Yoshimura M, van Leeuwen J, Shirahige K, Baryshnikova A, Brown G, Saito T, Costanzo M, Andrews B, Ohya Y, Osada H*, Yoshida M*, Myers CL*, **Boone C*** (2017). Functional annotation of chemical libraries across diverse biological processes. *Nat Chem Biol.* 13(9):982-993. doi:10.1038/nchembio.2436.
3. van Leeuwen J†, Pons C†, Mellor JC, Yamaguchi TN, Friesen H, Koschwanez J, Mattiazzi Ušaj M, Pechlaner M, Takar M, Ušaj M, VanderSluis B, Andrusiak K, Bansal P, Baryshnikova A, Boone C, Cao J, Cote A, Gebbia M, Horecka G, Horecka I, Kuzmin E, Legro N, Liang W, van Lieshout N, McNee M, San Luis B, Shaeri F, Shuteriqi E, Sun S, Yang L, Youn J, Yuen M, Costanzo M, Gingras A, Aloy P, Oostenbrink C, Murray A, Graham TR, Myers CL*, Andrews BJ*, Roth FP*, **Boone C*** (2016). Exploring genetic suppression interactions on a global scale. *Science* 354(6312) pii: aag0839

4. Costanzo M†, VanderSluis B†, Koch EN†, Baryshnikova A†, Pons C†, Tan G†, Wang W, Usaj M, Hanchard J, Lee SD, Pelechano V, Styles EB, Billmann M, van Leeuwen J, van Dyk N, Lin ZY, Kuzmin E, Nelson J, Piotrowski JS, Srikumar T, Bahr S, Chen Y, Deshpande R, Kurat CF, Li SC, Li Z, Usaj MM, Okada H, Pascoe N, San Luis BJ, SharifpoorS, Shuteriqi E, Simpkins SW, Snider J, Suresh HG, Tan Y, Zhu H, Malod-Dognin N, Janjic V, Przulj N, Troyanskaya OG, Stagljar I, Xia T, Ohya Y, Gingras AC, Raught B, Boutros M, Steinmetz LM, Moore CL, Rosebrock AP, Caudy AA, Myers CL*, Andrews B*, **Boone C*** (2016). A global genetic interaction network maps a wiring diagram of cellular function. *Science* 353(6306). pii: aaf1420.

Mapping Genetic Networks in Yeast and Human Cells

We’ ve generated a comprehensive genetic network in yeast cells, testing all possible 18 million gene pairs for genetic interactions. Negative interactions connected functionally related genes, mapped core bioprocesses, and identified pleiotropic genes, whereas positive interactions often mapped general regulatory connections among gene pairs, rather than shared functionality. The global network illustrates how coherent sets of genetic interactions connect protein complex and pathway modules to map a functional wiring diagram of the cell. To test whether the general principles of genetic networks are conserved, we are now utilizing CRISPR-Cas9 technology to conduct genome-wide screens and map genetic interactions in human cells.

Hiroyuki Sasanuma

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Education

2001 B.S. Genetics, Osaka, Osaka university, Supervisor: Prof. Hisao Masukata and Hideyuki Ogawa

2007 Ph.D. Genetics, Saitama, Saitama University, Supervisor: Prof. Kunihiro Ohta and Takehiko Shibata

Postdoctoral Training

2007-2008 The University of Tokyo, Graduate School of Arts and Sciences, College of Arts and Sciences, Advisor: Prof. Kuniro Ohta

2008-2011 Osaka University, Institute for Protein Research, Advisor: Prof. Akira Shinohara

Positions and Appointments

April 2011-April 2012 Assistant Professor, Kyoto University

April 2013-April 2021 Associate Professor, Kyoto University

April 2021-present Associate member, Group leader, Tokyo Metropolitan Institute of Medical Science,

April 2022-present Affiliate professor, Department of Chemistry, Tokyo Metropolitan University

Publication

1. A. Tomita, H. Sasanuma, T. Owa, Y. Nakazawa, M. Shimada, T. Fukuoka, T. Ogi, S. Nakada. Inducing multiple nicks promotes interhomolog

homologous recombination to correct heterozygous mutations in somatic cells. *Nat Commun*. 2023 Sep 15;14(1):5607.

2. D. Zong[#], S. Adam[#], Y. Wang[#], H. Sasanuma[#], E. Callen, A. Day, M. Kruhlak, N. Wong, M. Munro, A. Chaudhuri, B. Karim, X. Bing, S. Takeda, N. Johnson, D. Durocher, A. Nussenzweig, RNF168-Mediated Chromatin Ubiquitylation Is Redundant with BRCA1 in Homologous Recombination, *Molecular Cell*, 2019, 21;73(6):1267-1281.
3. H. Sasanuma, M. Tsuda, S. Morimoto, L. Saha, M. Rahman, Y. Kiyooka, H. Fujiike, A. Cherniack, J. Itou, E. Moreu, M. Toi, S. Nakada, H. Tanaka, K. Tsutsui, S. Yamada, A. Nussenzweig, and S. Takeda. BRCA1 ensures genome integrity by eliminating estrogen-induced pathological Topoisomerase II-DNA complexes *Proceeding of National Academy of Sciences*, 2018, 115 (45) E10642-E10651.
4. H. Sasanuma, K. Hirota, T. Fukuda, N. Kakusho, K. Kugou, Y. Kawasaki, T. Shibata, H. Masai, and K. Ohta. Cdc7-dependent phosphorylation of Mer2 facilitates initiation of yeast meiotic recombination. *Genes and Development* 2008 Feb 1;22(3):398-410.

BRCA1 promotes repair of estrogen-induced and topoisomerase II-dependent DNA double-strand breaks

Hiroyuki Sasanuma

BRCA1- and BRCA2-associated hereditary breast and ovarian cancer syndrome (HBOC) is characterized by an increased risk for female breast and ovarian cancer. It remains unclear why BRCA deficiency predominantly causes malignant tumors in estrogen-regulated tissues. BRCA1 is required for DSB repair by homologous recombination during the S/G2 phase. We found that, during the G1 phase, BRCA1-deficient MCF-7 cells exhibited marked increases in the amount of topoisomerase II (Top2) covalently bound to double-strand breaks (DSBs) (Top2 cleavage complex (Top2cc)) following exposure to 17β-estradiol (E2). These results indicate that BRCA1 plays a critical role in eliminating pathological Top2ccs induced by E2, independently of their functioning in homologous recombination. This genotoxicity of E2 was also observed in the mammary luminal epithelial cells of BRCA-deficient mice. Mechanistically, BRCA1 promotes the removal of Top2cc from DSB sites by recruiting MRE11 nuclease. This novel function of BRCA1 may help explain the female-organ-specific carcinogenesis of BRCA1-mutation carriers.

Marco Foiani

Biosketch

A molecular biologist by training, Marco Foiani is professor in Molecular Biology at the University of Milan and heads the genome integrity research lab at IFOM. Marco obtained his Ph.D is molecular and cell biology from University of Milan in 1988. in 1989, Marco moved to the lab of Dr. Alan Hinnebush at NIH-NICHD, Bethesda in the USA for his post-doctoral studies and remained there till 1991. In 1991, he returned to Italy to continue his research as an assistant professor at the University of Milan where he started his independent research career in the field of chromatin dynamics. He became associate professor and subsequently professor of molecular biology at the University of Milan in 1995 and 2001, respectively.

He was one of first scientists to join IFOM during its early gestational period. Genome integrity research lab started functioning in 2000 with the aim of studying the various pathways that safeguard genomic integrity and play a role in oncogenesis.

In 2009, Marco became the scientific director of IFOM. As the director, Marco was responsible for the research strategic planning, the development of programs aimed at translating basic research discoveries into practice and for the establishment of national and international co-operation programs and joint ventures. In 2009, he became vice-president of CEN, the European Center for Nanomedicine, a multi- and interdisciplinary approach promoted by IFOM and aimed at developing cutting-edge scientific research based on integration of knowledge from different fields such as biomedicine, physics, chemistry, computer science or engineering.

In 2012 he became the scientific director of COGENTECH, a benefit company owned by IFOM specializing in genetic cancer diagnostics. He has been a member of the editorial board of Cell (2009-2019), and is currently a member of the Academia Europea and EMBO.

His major contributions are within the fields of chromosome dynamics and genome integrity. His work has contributed to elucidate the ATR and ATM-dependent checkpoint processes controlling the interfaces between DNA replication, recombination, transcription and DNA topology and preventing abnormal chromosome transitions. In recent years, focus of his research has geared more towards the connections between cell metabolism and genome integrity pathways and between chromosome dynamics and mechano-transduction circuits controlling cell and nuclear plasticity.
https://www.ifom.eu/en/cancer-research/researchers/marco-foiani.php

Confined cell migration is genotoxic and oncogenic

Giulia Bastianello1-2, Gururaj Kidiyoor1, Conor Lowndes1, Qingsen Li1, Raoul Bonnal1, Fabio Iannelli1, Ramona Bason1, Fabrizio Orsenigo1, Mattia Pavani1, Andrea Ciliberto1, Valeria Cancila3, Claudio Tripodo1-3, Massimiliano Pagani1-2 and Marco Foiani1-2.

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3Department of Health Science, Tumor Immunology Unit, University of Palermo School of Medicine, Palermo, Italy. E-mail: marco.foiani@ifom.eu

Chromosomal instability (CIN) and aneuploidy are hallmarks of cancer, driving intra-tumoral heterogeneity and phenotypic adaptation in complex micro-environments. Distant metastases often acquire novel mutations not seen in the primary tumor, suggesting interstitial migration in metastatic cells is a genotoxic process. We combined live cell imaging, micro-fluidic approaches and scRNA-seq to investigate the impact of mechanical stress generated during confined migration on genetic heterogeneity in cancer cells. We found that tumor cells dividing across constrictions exhibit altered spindle pole organization, chromosome mis-segregations and micronuclei formation. Mechanical stress leads to gene copy number variations due to defective mitosis; moreover, migration across constrictions up-regulates c-MYC oncogenic transcriptional signature via c-MYC locus amplifications. Altogether our data demonstrate that mechanical stress during metastatic migration is a source of mitotic stress, CIN, aneuploidy and c-MYC amplification.

Frank Uhlmann

The Francis Crick Institute, London, UK

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EDUCATION & DEGREE

1994 Diploma, Biochemistry & Physiological Chemistry, University of Tübingen
1997 PhD, Memorial Sloan-Kettering Cancer Center / University of Tübingen Postdoc, Institute of Molecular Pathology (I.M.P.), Vienna

EMPLOYMENT

2000 Research Scientist, Imperial Cancer Research Fund (ICRF), London
2005 Senior Group Leader, Cancer Research UK London Research Institute
2015 Principal Group Leader, The Francis Crick Institute, London
2017~ Visiting Professor, Tokyo Institute of Technology

EDITORIAL ACTIVITIES

Associate Editor, Chromosoma (2003~)
Editorial Board, EMBO Journal (2011~)
BMC Biology (2009~)
Guest Editor, Current Opinion in Cell Biology (2010)
PLoS Biology and PLoS Genetics (2013~)

AWARDS

2015: Fellow of the Royal Society

2006: EMBO Gold Medal
2006: EMBO Member
2005: Hooke Medal of the British Society for Cell Biology
2003: Balfour Lecturer of the Genetics Society
2002: EMBO Young Investigator

RECENT PUBLICATIONS

1. Uhlmann, F. *Nat. Rev. Mol. Cell Biol.* 17, 399-412. (2016)
2. Kakui, Y. et al. *Nat. Genet.* 49, 1553-1557 (2017)
3. Murayama, Y. et al. *Cell* 172, 465-477 (2018)
4. Liu, H. et al. *Mol. Cell* 78, 725-738 (2020)
5. Higashi, T. L. et al. *Mol. Cell* 79, 917-933 (2020)
6. Muñoz, S. et al. *Nat. Commun.* 13, 7698 (2022)
7. Minamino, M. et al. *Cell* 186, 837-849. (2023)

To know your sister: The establishment of sister chromatid cohesion during DNA replication

Sam Glaser1,2, Masashi Minamino1, Sudikchya Shrestha1, John Diffley2, Frank Uhlmann1

1Chromosome Segregation Laboratory and 2Chromosome Replication Laboratory

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Concomitant with DNA replication, the ring-shaped chromosomal cohesin complex establishes cohesion between newly replicated sister chromatids, which forms the basis for their faithful segregation during cell divisions. Sister chromatid cohesion establishment encompasses two events. First, cohesin transitions from entrapping one DNA before replication to co-entrapping two DNAs, the two sister chromatids. Secondly, two conserved lysine residues on the Smc3 cohesin subunit must be acetylated at the time of DNA replication to stabilize cohesin on DNA, thus ensuring enduring sister chromatid cohesion. We will report on our ongoing biochemical and single-molecule approaches to investigate and visualize replisome-cohesin encounters. Our results paint a picture in which replication-associated DNA structures and proteins not only serve to duplicate DNA, but also participate in an active process that results in the establishment of stable sister chromatid cohesion establishment during DNA replication.

Dirk Remus, Ph.D.

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EDUCATIONAL BACKGROUND

2003 PhD Biochemistry, University of Heidelberg / University of California, Berkeley, Advisor: Prof. Mike Botchan

1999 Diploma (MS), Molecular Biology University of Heidelberg, Germany

RESEARCH EXPERIENCE

2005–2010 Postdoctoral Fellow, Cancer Research UK, London Research Laboratories, Clare Hall Laboratory of John Diffley, Ph.D.

2003–2005 Postdoctoral Fellow, University of California, Berkeley Department of Molecular and Cell Biology Laboratory of Prof. Mike Botchan

1999–2003 Graduate Student, University of California, Berkeley Department of Molecular and Cell Biology Laboratory of Prof. Mike Botchan

PROFESSIONAL POSITIONS & EMPLOYMENT

2020–present Member, Molecular Biology Program Memorial Sloan Kettering Cancer Center

2020–present Professor, Weill Cornell Graduate School of Medical Sciences Cornell University

2020–present Professor, Gerstner Sloan Kettering Graduate School of Biomedical Sciences Memorial Sloan Kettering Cancer Center

2016–2020 Associate Membe,r Molecular Biology Program Memorial Sloan Kettering Cancer Center

2016–2020 Associate Professor, Weill Cornell Graduate School of Medical Sciences Cornell University
2016–2020 Associate Professor, Gerstner Sloan Kettering Graduate School of Biomedical Sciences Memorial Sloan Kettering Cancer Center
2010–2016 Assistant Member Molecular Biology Program Memorial Sloan Kettering Cancer Center
2010–2016 Assistant Professor, Weill Cornell Graduate School of Medical Sciences Cornell University
2010–2016 Assistant Professor, Gerstner Sloan Kettering Graduate School of Biomedical Sciences Memorial Sloan Kettering Cancer Center

HONORS, AWARDS
2011–2014 Louis V. Gerstner, Jr. Young Investigator Award
2006–2008 EMBO Long Term Fellowship
2005–2010 CRUK postdoctoral fellowship

ACADEMIC SERVICE
2022-present Weill Cornell Graduate School of Medical Sciences BCMB PhD program, Director
2020-present GSK Curriculum committee, Experimental Biology
2014–2022 Weill Cornell BCMB core course, Director.

GRANT REVIEW AND STUDY SECTIONS
NIH Study Sections
2021 – 2025 Molecular Genetics (MG), member
03/2021 Fellowships: Genes, Genomes, and Genetics, ad hoc
10/2020 Molecular Genetics A (MGA), ad hoc
02/2020 Molecular Genetics A (MGA), ad hoc
11/2018 Fellowships: Genes, Genomes, and Genetics, ad hoc

Additional
2020 Boehringer Ingelheim Foundation, PhD fellowhips
2019 Wellcome Trust Investigator award, UK
2018 National Science Foundation

2018 Boehringer Ingelheim Foundation, PhD fellowhips
2016 Dutch Foundation for Fundamental Research on Matter, FOM
2016 National Science Foundation
2015 Boehringer Ingelheim Foundation, PhD fellowhips
2012 Boehringer Ingelheim Foundation, PhD fellowhips
2010 Biotechnology and Biological Sciences Research Council, UK

AD HOC REVIEWER

Nature, Cell, Nature Structural and Molecular Biology, Molecular Cell, Genes & Development, Structure, Cell Reports, EMBO Journal, EMBO Reports, eLife, Nucleic Acids Research, Nature Communications, Science Advances, Journal of Biological Chemistry, Plos Genetics, Journal of Molecular Biology

PUBLICATIONS

Primary Research Papers

1. Schrecker M, Castaneda JC, **Remus D***, Hite R*. Multistep loading of a DNA sliding clamp onto DNA by replication factor C. *eLife*. 2022 Aug 8; 11:e78253. DOI: 10.7554/eLife.78253. PMID:35939393. PMCID: PMC9359705. *co-corresponding author.
2. Regan-Mochrie G, Hoggard T, Bhagwat N, Lynch G, Hunter N, **Remus D**, Fox C, Zhao X. Yeast ORC sumoylation status fine-tunes origin licensing. *Genes & Development*. 2022 Aug 4. Doi:10.1101/gad.349610.122. PMID: 35926881.
3. Chen T, Alcorn H, Devbhandari S, *Remus D*, Lacy E, Huangfu D. Anderson KV. A hypomorphic mutation in Pold1 disrupts the coordination of embryo size expansion and morphogenesis during gastrulation. *Biol Open*. 2022 Aug 15; 11(8): bio059307. doi: 10.1242/bio.059307. PMID:35876795. PMCID: PMC9382117.
4. Scherr M, Abd Wahab S, **Remus D**, Duderstadt KE. Born to slide: Mobile origin licensing factors confer resistance to transcription conflicts. *Cell Reports* 2022; Mar 22; 38(12):110531. doi: https//doi.org/10.1016/j.celrep.2022.110531. PMID: 3532078. PMCID: PMC8961423.
5. Castaneda JC, Schrecker M, **Remus D***, Hite RK*. Mechanisms of loading and release of the 9-1-1 checkpoint clamp. *Nature Structural and*

Molecular Biology 2022; doi: 10.1038/s41594-022-00741-7. PMID 35314831. *corresponding author

6. Philip J, Ord M, Silva A, Singh A, Diffley JFX, **Remus D**, Loog M, Ikui AE. Cdc6 is sequentially regulated by PP2A-Cdc55, Cdc14 and Sic1 for origin licensing in S. cerevisiae. *eLife* 2022; Feb 10; 11:e74437. doi: 10.7554/eLife.74437. PMID: 35142288.
7. Kumar S, Batra S, Griffith JD, **Remus D**. The interplay of RNA:DNA hybrid structure and Gquadruplexes determines the outcome of R-loop-replisome collisions. eLife 2021; Sep 8;10:e72286. doi: 10.7554/eLife.72286. PMID: 34494544.
8. Abd Wahab S and **Remus D**. Antagonistic control of DDK binding to licensed replication origins by Mcm2 and Rad53. *eLife* 2020; Jul 23;9:e58571.
9. Meng X, Wei L, Devbhandari S, Zhang T, Xiang J, **Remus D**, Zhao X. The Pol e catalytic core plays a structural role in replisome assembly and relies on a unique domain for efficient strand synthesis. *Nature Communications* 2020; 11(1):2437. PMID 32415104.
10. Devbhandari S and **Remus D**. Rad53 limits CMG helicase uncoupling from DNA synthesis at replication forks. *Nature Structural and Molecular Biology* 2020; 27(5):461-471. PMID32341532.
11. Gan H, Yu C, Devbhandari S, Sharma S, Han J, Chabes A, **Remus D**, Zhang Z. Checkpoint kinase Rad53 couples leading- and lagging-strand DNA synthesis under replication stress. *Molecular Cell* 2017; 68: 446-455.
12. Frigola J, He J, Kinkelin K, Pye VE, Renault L, Douglas ME, **Remus D**, Cherepanov P, Costa A, Diffley JFX. Cdt1 stabilizes an open MCM ring for helicase loading. *Nature Communications* 2017; 8: 15720
13. Devbhandari S, Jiang J, Kumar C, Whitehouse I, and **Remus D**. Chromatin constrains the initiation and elongation of DNA replication. *Molecular Cell* 2017; 65: 131-141
14. Gros J, Kumar C, Lynch G, Yadav T, Whitehouse I & **Remus D**. Post-licensing Specification of Eukaryotic Replication Origins by Facilitated Mcm2-7 Sliding along DNA. *Molecular Cell* 2015;60: 797-807.
15. Gros J, Devbhandari S & **Remus D**. Origin plasticity during budding yeast DNA replication in vitro. *EMBO J* 2014; 33: 621-36.
16. Frigola J, **Remus D**, Mehanna A & Diffley JF. ATPase-dependent quality

control of DNA replication origin licensing. *Nature* 2013; 495: 339-43.
17. **Remus D**, Beuron F, Tolun G, Griffith JD, Morris EP & Diffley JF. Concerted loading of Mcm2-7 double hexamers around DNA during DNA replication origin licensing. *Cell* 2009; 139: 719-30.
18. **Remus D**, Blanchette M, Rio DC & Botchan MR. CDK phosphorylation inhibits the DNA-binding and ATP-hydrolysis activities of the Drosophila origin recognition complex. *J Biol Chem* 2005; 280: 39740-51.
19. **Remus D**, Beall EL & Botchan MR. DNA topology, not DNA sequence, is a critical determinant for Drosophila ORC-DNA binding. *EMBO J* 2004; 23: 897-907.
20. Chesnokov I, **Remus D** & Botchan, M. Functional analysis of mutant and wild-type Drosophila origin recognition complex. *Proc Natl Acad Sci U S A* 2001; 98: 11997-2002.
21. Chesnokov I, Gossen M, **Remus D** & Botchan M. Assembly of functionally active Drosophila origin recognition complex from recombinant proteins. *Genes Dev* 1999; 13: 1289-96.

REVIEWS, BOOK CHAPTERS AND COMMENTARIES

1. Kumar C, **Remus D**. Looping out of control: R-loops in transcription-replication conflict. *Chromosoma*. 2023; doi: 10.1007/s00412-023-00804-8. PMID: 37419963.
2. Kumar C, **Remus D**. A transcription-based approach to purify R-loop-containing plasmid DNA templates in vitro. *STAR Protocols*. 2022; 4(1):101937. Doi: 10.1016/j.xpro.2022.101937. PMID:36520635. PMCID: PMC9758483.
3. Batra S, Devbhandari S, **Remus D**. CMG helicase activity on G4-containing templates. *Methods Enzymol*. 2022; 672:233-260. Doi 10.1016/bs.mie.2022.02.020. Epub 2022 Mar 25. PMID:35934477.
4. Kumar C & **Remus D**. Eukaryotic replication origins: Strength in flexibility. *Nucleus*. 2016. 7:292-300.
5. **Remus D**. The role of Mcm2-7 in replication initiation. *In The Initiation of DNA Replication in Eukaryotes*. 2016. Springer. DOI 10.1007/978-3-319-24696-3
6. **Remus D** & Diffley JF. Eukaryotic DNA replication control: lock and load, then fire. *Curr Opin Cell Biol* 2009; 21: 771-7.

Biochemical analysis of programmed and unscheduled eukaryotic replication fork stall events

Chromosomes are replicated by large multi-subunit protein complexes, called replisomes. In eukaryotes, replisomes are organized around the replicative DNA helicase, CMG (Cdc45-MCM-GINS), which encircles and translocates in 3' to 5' direction along the leading strand template to mediate DNA unwinding at the replication fork. To ensure the faithful and complete replication of the genome, replisomes have to contend with the highly complex chromatin landscape of eukaryotic chromosomes and other potential obstacles, such as DNA damage and non-B DNA secondary structures, which may impede the replicative DNA polymerases or the helicase and, consequently, threaten genome integrity. While some obstacles to replication forks may occur in a stochastic or 'unscheduled' manner across the genome, certain genomic loci induce a 'programmed' and stable stalling of replication forks, for example, to limit transcription-replication conflict by co-orienting transcription and replication.

Here, I will present data from our lab on the biochemical characterization of both programmed and unscheduled fork stall events using fully reconstituted yeast and human replisomes. For this, I will focus on our work on the characterization of replisome collisions with G-quadruplexes (G4s) or the rDNA replication fork barrier (RFB) as examples for unscheduled and programmed fork stall events, respectively. The data highlight the distinct molecular determinants for both types of fork stalls.

Daochun Kong

Professor
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Degrees

1983	B.S. Lanzhou University
1989	M.S. Shanghai Institute of Biochemistry, Chinese Academy of Science
1995	Ph.D. Temple University Medical School, USA.

Professional Training

1979-1983	Lanzhou University
1986-1989	Graduate Student, Shanghai Institute of Biochemistry, Chinese Academy of Science
1990.11-1994.10	Graduate Student, Department of Biochemistry, Temple University Medical School
1994.11-1997.7	Postdoctoral Fellow, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School
1997.7-1997.12	Postdoctoral Fellow, Department of Genetics and Molecular Biology, Medical School, Johns Hopkins University,
1998,1-2000	Postdoctoral Fellow, The Memorial Sloan-Kettering Cancer Center

Employment

1983-1986	Research Fellow, Lanzhou Institute of Biomedicine, China
1989-1990	Head, The Group of Protein Purification, Shanghai Academy of Biomedicine, China

Academic Appointments

2000-2005	Research scientist, National Institutes of Health, U.S.A
2005-	Professor, The National Laboratory of Protein Engineering

and Plant Genetic Engineering, College of Life Sciences, Peking University, China

2012- Investigator, Peking-Tsinghua Center for Life Sciences

Major Research Interest

Metabolism of nucleic acids: DNA replication, replication fork stability, repair of DNA double-strand breaks, checkpoint control, cell cycle control, chromatin structures.

Selected Publications

1. Xizhou Li, Lu Wang, Xiaoqin Liu, Zeqi Zheng, **Daochun Kong***. Cellular regulation and stability of DNA replication forks in eukaryotic cells. *DNA Repair* (Amst).2022 Oct 10;120:103418. doi: 10.1016/j.dnarep.2022.103418.
2. Sijie Liu#*, Xizhou Li#, Xiaoqin Liu#, Jingna Wang, Lingyan Li, and **Daochun Kong***, RNA polymerase III directly participates in DNA homologous recombination. *Trends Cell Biol.* 2022 Jul 7:S0962-8924(22)00146-5. doi: 10.1016/j.tcb.2022.06.007.
3. Yang Liu#, Lu Wang#, Xin Xu, Yue Yuan, Bo Zhang, Zeyang Li, Yuchen Xie, Rui Yan, Zeqi Zheng, Jianguo Ji, Johanne M. Murray, Antony M. Carr, **Daochun Kong**. The intra-S phase checkpoint directly regulates replication elongation to preserve the integrity of stalled replisomes. *Proc Natl Acad Sci U S A.* 2021 Jun 15;118(24):e2019183118. doi: 10.1073/pnas.2019183118.
4. Sijie Liu#, Yu Hua#, Jingan Wang#, Lingyan Li#, Junjie Yuan, Bo Zhang, Ziyang Wang, Jianguo Ji, **Daochun Kong**. RNA polymerase III is required for the repair of DNA double-strand breaks by homologous recombination. *Cell*. 2021 Mar 4;184(5):1314-1329.e10. doi: 10.1016/j.cell.2021.01.048. Epub 2021 Feb 23.
5. Gang Feng#, Yue Yuan#, Zeyang Li, Lu Wang, Bo Zhang, Jiechen Luo, Jianguo Ji, **Daochun Kong**. Replication fork stalling elicits chromatin compaction for the stability of stalling replication forks. *Proc Natl Acad Sci U S A.* 2019 Jul 16;116(29):14563-14572. doi: 10.1073/pnas.1821475116. Epub 2019 Jul 1.
6. Ling Guan, Peng He, Fang Yang, Yuan Zhang, Yunfei Hu, Jienv Ding, Yu Hua, Yi Zhang, Qiong Ye, Jiazhi Hu, Tao Wang, Changwen Jin, **Daochun**

Kong, Sap1 is a replication initiation factor essential for the assembly of pre-replicative complex in the fission yeast Schizosaccharomyces pombe. *J Biol Chem*. 2017, 292: 6056-6075.

7. Bochao Liu, Jiazhi Hu, Jingna Wang, **Daochun Kong**, Direct Visualization of RNA-DNA Primer Removal from Okazaki Fragments Provides Support for Flap Cleavage and Exonucleolytic Pathways in Eukaryotic Cells. *J Biol Chem*. 2017, 292: 4777-4788.
8. Jiazhi Hu, Lei Sun, Fenfen Shen, Yufei Chen, Yu Hua, Yang Liu, Mian Zhang, Yiren Hu, Qingsong Wang, Wei Xu, Fei Sun, Jianguo Ji, Johanne M. Murray, Antony M. Carr, and **Daochun Kong**. The intra-S phase checkpoint targets Dna2 to prevent stalled replication forks from reversing. *Cell*. 2012 Jun 8;149(6):1221-32.

Cellular Regulation and Stability of DNA Replication Forks in Eukaryotes

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College of Life Sciences, Peking University

A human genome harbors approximately 106 - 107 natural DNA replication fork barriers, which results in frequent replication fork stalling. Stalled replication forks tend to collapse, potentially leading to incomplete DNA replication and gross genomic alterations. Fortunately, eukaryotic cells have developed a sophisticated regulation system to stabilize stalled replication forks. The intra-S checkpoint is an essential system to prevent stalled replication forks from collapsing. In the last decade, considerable progress has been made in understanding the mechanism of how the checkpoint regulates to stabilize stalled replication forks; however, some of key mechanisms still remain to be uncovered. Following the identification of the checkpoint target Dna2 and the replicative helicase CMG complex, we recently demonstrated that the ubiquitin E3 ligase Brl2 is regulated by the intra-S checkpoint in response to replication fork stalling. When replication forks stall, Cds1Chk2 phosphorylates Brl2 at five serine residues, which results in marked reduction of H2BK119ub1 in the chromatin regions around stalled replication forks. Both brl2-5D (with the five serine residues mutated to aspartic acid) and htb1-K119R drastically reduce the sensitivity of cds1Chk2Δ or rad3ATRΔ cells to hydroxyurea. In addition, both brl2-5D and htb1-K119R result in remarkable chromatin condensation around stalled forks, which prevents physical separation of the replicative helicase CMG and DNA polymerases, thereby preserving replisome integrity and stabilizing stalled replication forks. Thus, this finding demonstrates that nucleosomes are a critical target of the intra-S checkpoint. Furthermore, this finding indicates that the intra-S checkpoint plays an critical role in establishing heterochromatin and condensed chromatin domains.