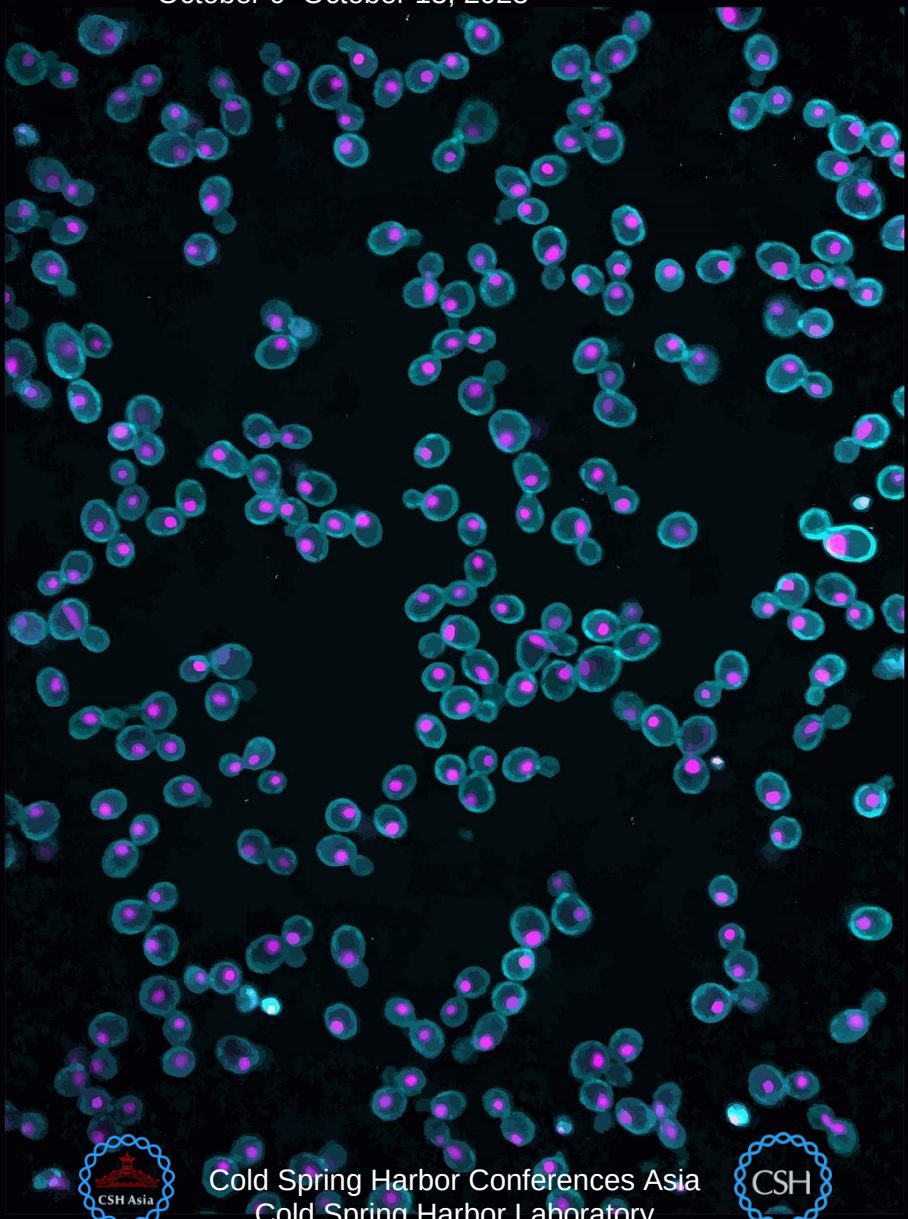
Abstracts of papers presented
at the 2023 Cold Spring Harbor Asia Conference
*Matsue, Japan*

# YEAST AND LIFE SCIENCES

October 9–October 13, 2023



Cold Spring Harbor Conferences Asia
Cold Spring Harbor Laboratory

Abstracts of papers presented
at the 2023 Cold Spring Harbor Asia Conference
Matsue, Japan

# YEAST AND LIFE SCIENCES

October 9–October 13, 2023

Arranged by

Brenda J. Andrews, *University of Toronto*
Marco Foiani, *The FIRC Institute of Molecular Oncology*
Daochun Kong, *Peking University*
Hisao Masai, *Tokyo Metropolitan Institute of Medical Science*
Frank Uhlmann, *The Francis Crick Institute*

Cold Spring Harbor Conferences Asia
Cold Spring Harbor Laboratory

**CSH-Asia would like to thank the following for their generous support of this meeting**


---

*Cover image credit*: Alex Daiejavad, Andrews and Boone Labs, University of Toronto, Canada.

**YEAST AND LIFE SCIENCES (*Matsue, Japan)***
Monday, October 9– Friday, October 13, 2023

| | | |
|---|---|---|
| October 9<br>Monday | 1:00 pm<br>2:00 pm<br>4:00 pm<br>6:50 pm | *Registration*<br>*Tea Ceremony*<br>*Reception and Cocktails*<br>*Orientation* |
| October 9<br>Monday | 7:00 pm – 9:00 pm | 1 Cell Biology: Organelle Interactions and Dynamics |
| October 9<br>Monday | 9:00 pm – 10:00 pm | *Social Hour* |
| October 10<br>Tuesday | 9:00 am – 12:00 pm | **2** DNA Replication and Repair |
| October 10<br>Tuesday | 1:30 pm – 3:00 pm | **3** Chromosome Dynamics and Regulation |
| October 10<br>Tuesday | 3:00 pm – 4:00 pm | *Tea Ceremony* |
| October 10<br>Tuesday | 4:00 pm – 5:20 pm | **3 (continued)**<br>Chromosome Dynamics and Regulation |
| October 10<br>Tuesday | 5:30 pm – 7:00 pm | **4** Poster Flash Talks |
| October 10<br>Tuesday | 7:30 pm – 9:30 pm | *Dinner* |
| October 11<br>Wednesday | 9:00 am – 12:00 pm | **5** More Chromosome and Chromatin Biology |
| October 11<br>Wednesday | 12:30 pm | *Excursion* |
| October 11<br>Wednesday | 5:30 pm | *Dinner* |
| October 11<br>Wednesday | 7:00 pm – 10:00 pm | **Poster Session** |
| October 11<br>Wednesday | 9:00 pm – 10:00 pm | *Social Hour* |

*Continued on next page*

| | | |
|---|---|---|
| October 12 Thursday | 9:00 am – 12:00 pm | **6** Insights from Functional Genomics and Synthetic Biology |
| October 12 Thursday | 1:30 pm – 5:30 pm | **7** Signaling, Stress Response and Gene Regulation |
| October 12 Thursday | 6:30 pm – 9:00 pm | **Cocktails and Banquet** |
| October 12 Thursday | 9:30 pm – 11:30 pm | **Final Mixer** |
| October 13 Friday | 9:00 am – 12:00 pm | **8** Bioprocess Relationships (and unexpected observations) |

**Kunibiki Messe (Shimane Prefectural Convention Center)**

*Meeting venue:* International Conference Hall, 3rd floor of the Convention Center
*Poster session:* International Conference Hall, 3rd floor of Convention Center

*CSHA office:* Room 301

*Reception and Cocktails*: Small Hall, first floor of Convention Center
*Tea Ceremony (Oct. 9 and 10):* International Conference Foyer, 3rd floor of Convention Center
*Social Hour (Oct. 9 and 11):* International Conference Hall and Foyer, 3rd floor of Convention Center
*Lunch (Oct. 10 and 12):* Small Hall, first floor of Convention Center
*Dinner (Oct.10 and 11):* Small Hall, first floor of Convention Center
*Excursion (Oct.11):* The Adachi Museum of Art
*Banquet (Oct.12):* Yuushien Garden Restaurant.
*Final Mixer (Oct.12):* WindsorKnot

Abstracts are the responsibility of the author(s) and publication of an abstract does not imply endorsement by Cold Spring Harbor Asia of the studies reported in the abstract.

These abstracts should not be cited in bibliographies. Material herein should be treated as personal communications and should be cited as such only with the consent of the author.

Please note that ANY photography or video/audio recording of oral presentations or individual posters is strictly prohibited except with the advance permission of the author(s), the organizers, and Cold Spring Harbor Asia.

PROGRAM

MONDAY, October 9

*Reception / Orientation*

MONDAY, October 9—7:00 PM

**SESSION 1** CELL BIOLOGY: ORGANELLE INTERACTIONS AND DYNAMICS

**Chairperson:** **Daochun Kong,** Peking University, Beijing, China

**KEYNOTE SPEAKER**

**Reflections on my autophagy research with yeast**
Yoshinori Ohsumi [30'+10']
Presenter affiliation: Tokyo Institute of Technology, Yokohama,Kanagawa, Japan. 1

**Exocytic plasma membrane flows remodel endoplasmic reticulum-plasma membrane tethering for septin collar assembly**
Shinju Sugiyama, Keiko Kono [20'+5']
Presenter affiliation: Okinawa Institute of Science and Technology Graduate University, Onna, Okinawa, Japan. 2

**VAP-mediated membrane tethering mechanisms implicate ER-PM contact function in pH homeostasis**
Karling Hoh, Baicong Mu, Tingyi See, Amanda Ng, Annabel Ng, Dan Zhang [20'+5']
Presenter affiliation: Temasek Life Sciences Laboratory, Singapore; National University of Singapore, Singapore. 3

**Yeast lunapark regulates formation of trans-atlastin complexes for ER membrane fusion**
Eunhong Jang, So Young Yoon, Naho Ko, Minseok Kim, Gongmin Na, Youngsoo Jun [10'+5']
Presenter affiliation: Gwangju Institute of Science and Technology, Gwangju, South Korea. 4

**The exocyst complex is involved in autophagosome biogenesis in *Saccharomyces cerevisiae***
Ruchika Kumari, Sunaina Singh, Ravi Manjithaya [10'+5']
Presenter affiliation: Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India. 5

TUESDAY, October 10—9:00 AM

**SESSION 2** DNA REPLICATION AND REPAIR

**Chairpersons:** **Hisao Misai,** Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
**Dirk Remus,** Memorial Sloan-Kettering Cancer Center, New York, USA

**Checkpoint targets the ubiquitin E3 ligase Brl2 to regulate chromatin structures in response to replication fork stalling**
Xiaoqin Liu, Bo Zhang, Yu Hua, Xizhou Li, Daochun Kong [20'+5']
Presenter affiliation: Peking University, Peking-Tsinghua Center for Life Sciences, Beijing, China. 6

**Structural basis for eukaryotic replisome stalling at G-quadruplexes**
Sahil Batra, Benjamin Allwein, Charanya Kumar, Richard Hite, Dirk Remus [20'+5']
Presenter affiliation: Memorial Sloan Kettering Cancer Center, New York, New York. 7

**Post-replicative accumulation of Rad51 is resolved by Rad54 in *S. pombe***
Goki Taniguchi, Hiroshi Iwasaki, Hideo Tsubouchi [10'+5']
Presenter affiliation: Tokyo Institute of Technology, Yokohama, Japan. 8

***Break***

**G4 binding and oligomerization activities of fission yeast Rif1 protein are required for long-range regulation of origin firing**
H. Masai, Y. Kanoh, D. Kohda, T. Sagi [20'+5']
Presenter affiliation: Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan. 9

TUESDAY, October 10—1:30 PM

**SESSION 3** CHROMOSOME DYNAMICS AND REGULATION

**Chairpersons:** **Marco Foiani,** IFOM The FIRC Institute of Molecular Oncology, Milan, Italy
**Xiangwei He,** Zhejiang University, Hangzhou, China

**PCNA recruits cohesin loader Scc2/NIPBL to ensure sister chromatid cohesion**
Ivan Psakhye, Ryotaro Kawasumi, Takuya Abe, Kouji Hirota, Dana Branzei [10'+5']
Presenter affiliation: IFOM ETS, the AIRC Institute of Molecular Oncology, Milan, Italy. 16

TUESDAY, October 10—3:00 PM

**Tea Ceremony**

***SESSION 3, continued***

**3D genome organization in fission yeast**
Ken-ichi Noma, Osamu Iwasaki, Hideki Tanizawa, Sanki Tashiro [20'+5']
Presenter affiliation: University of Oregon, Eugene, Oregon; Hokkaido University, Sapporo, Japan. 17

**Sch$^{9S6K}$ -mediated control of DNA repair and DNA damage response during chronological aging**
Elisa Ferrari, Chiara Lucca, Ghadeer Shubassi, Marco Foiani [20'+5']
Presenter affiliation: IFOM The FIRC Institute of Molecular Oncology, Milan, Italy. 17.5

**Condensin-mediated chromatin loop formation for controlling mitotic chromosome structure**
Yasutaka Kakui, Christopher Barrington, Frank Uhlmann [10'+5']
Presenter affiliation: Waseda University, Tokyo, Japan. 18

TUESDAY, October 10—5:30 PM

**SESSION 4** POSTER FLASH TALKS

**Chairpersons:** **Hisao Masai,** Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

**A fission yeast-based platform for screening potent human kinesin-5/Eg5 inhibitors**
Fara Difka Afdilla, Masashi Yukawa
Presenter affiliation: Hiroshima University, Higashi-Hiroshima, Japan. 19

**SESSION 5** MORE CHROMOSOME AND CHROMATIN BIOLOGY

**Chairpersons:** **Fred Chang,** University of California, San Francisco, San Francisco, California, USA
**Qing Li,** Peking University, Beijing, China



***Break***

**KEYNOTE SPEAKER**

WEDNESDAY, October 11—12:00 PM

***Excursion***

WEDNESDAY, October 11—7:00 PM

**POSTER SESSION**

***See Poster Flash Talks (p. viii – xii) for List of Posters***

THURSDAY, October 12—9:00 AM

**SESSION 6** INSIGHTS FROM FUNCTIONAL GENOMICS AND SYNTHETIC BIOLOGY

**Chairpersons:** **Charlie Boone,** University of Toronto, Toronto, Canada
**Smezhana Oliferenko,** The Francis Crick Institute, London, United Kingdom



***Break***

THURSDAY, October 12—1:30 PM

**SESSION 7** SIGNALING, STRESS RESPONSE AND GENE REGULATION

**Chairpersons:** **Mart Loog,** University of Tartu, Tartu, Estonia
**Gloria Brar,** University of California, Berkeley, Berkeley, California, USA

**The role of histone H3 lysine 4 methylation in maintaining redox homeostasis under endoplasmic reticulum stress**
Chia-Ling Hsu, Wei-Hsun Hsiao, Tzu-Hsin Ku, Cheng-Fu Kao [10'+5']
Presenter affiliation: Academia Sinica, Taipei, Taiwan. 65

**Pib2 is a cysteine sensor involved in TORC1 activation**
Qingzhong Zeng, Yasuhiro Araki, Takeshi Noda [10'+5']
Presenter affiliation: Osaka University, Osaka, Japan. 66

***Break***

**Translation factor eIF5A is the sensor and effector for autoregulation of cellular polyamines**
Thomas E. Dever, Ivaylo P. Ivanov, Byung-Sik Shin, Arya Vindu, Chune Cao, Joo-Ran Kim [20'+5']
Presenter affiliation: National Institutes of Health, Bethesda, Maryland. 67

**Off-target repression by genomic cassette insertion masks true mutant phenotypes**
Emily Powers, Charlene Chan, Gloria Brar [20'+5']
Presenter affiliation: UC Berkeley, Berkeley, California. 68

**Identification of novel quality control factors at the TOM complex**
Arushi Gupta, Jiyao Song, Jeannine Engelke, Swadha Mishra, Fabian den Brave, Thomas Becker [10'+5']
Presenter affiliation: University of Bonn, Bonn, Germany. 69

**Acidic growth conditions stabilize the ribosomal RNA gene cluster and extend lifespan**
Yo Hasegawa, Hiroyuki Ooka, Tsuyoshi Wakatsuki, Mariko Sasaki, Ayumi Yamamoto, Takehiko Kobayashi [10'+5']
Presenter affiliation: The University of Tokyo, Tokyo, Japan. 70

THURSDAY, October 12—6:30 PM

**COCKTAILS and BANQUET**

**SESSION 8** BIOPROCESS RELATIONSHIPS (AND UNEXPECTED OBSERVATIONS)

**Chairpersons:** **Frank Uhlmann,** The Francis Crick Institute, London, UK United Kingdom
**Makoto Kawamukai,** Shimane University, Matsue, Japan



**Concluding Remarks**

# REFLECTIONS ON MY AUTOPHAGY RESEARCH WITH YEAST

Yoshinori Ohsumi

Tokyo Institute of Technology, Institute of Innovative Research,
Yokohama,Kanagawa, Japan

I have been studying autophagy in budding yeast for more than 34 years. In the meantime, this intracellular degradation pathway has become one of the major topics of cell biology and has made remarkable progress. Today, I would like to briefly describe how I came across and studied the molecular details of autophagy in yeast, and give an opportunity to share my thoughts on the role that yeast has played, the current status of research, and future perspective.

I had been interested in vacuolar function and had discovered that yeast vacuoles function as storage compartment of amino acids and ions, then in 1988 I wanted to pursue the possibility that vacuoles function as sites of intracellular degradation. A clue to this conundrum came from simple light microscopic observation of vacuolar proteinase deficient cells starved of nitrogen sources. Electron microscopic analysis revealed that the process was essentially identical to the membrane dynamics of autophagy previously known in animal cells. The most important feature of the yeast system is that the progression of intracellular membrane phenomena can be observed in real time under a light microscope. Autophagy involves the unique de novo membrane biogenesos, and its mechanism and physiological functions had long remained unknown, with no means of analysis other than morphological analysis by electron microscopy. The yeast system was the first to apply genetics on autophagy, enabling molecular biological dissection. As a result, a set of ATG genes essential for autophagy was identified. Surprisingly, they were almost all novel genes whose functions were unknown in yeast, where many issues had been analyzed. Subsequently, the structure and function of the Atg proteins encoded by these genes were elucidated. It became clear that the 18 essential ATG genes constitute six functional units that assemble sequentially on the vacuolar membrane and are involved in autophagosome formation.

On the other hand, these yeast ATG genes were shown to be conserved in animals and plants, indicating that autophagy was acquired early in the evolution of eukaryotic cells. The identification of these genes has revolutionized autophagy research to date. Autophagy have been studied using various organisms, cells, organs and individuals, revealing so diverse physiological functions. Two major functions are mentioned: autophagy is involved in the recycling of nutrient sources during starvation, and also in quality control, which removes unwanted or dangerous substances from the cytoplasm and keeps the inside of the cell clean at all times. Although autophagy was previously thought to randomly degrade cytoplasm, selective autophagy, which selectively degrades organelles and other substances, is now becoming better understood. Currently, there is much interest in autophagy as a mechanism for suppressing neurodegenerative diseases and cancer, as well as for maintaining health and longevity.

However, molecular details and physiological significance of autophagy still remain unclear, and further biochemical studies are needed. We would like to present and discuss our recent results.

# EXOCYTIC PLASMA MEMBRANE FLOWS REMODEL ENDOPLASMIC RETICULUM-PLASMA MEMBRANE TETHERING FOR SEPTIN COLLAR ASSEMBLY

Shinju Sugiyama, Keiko Kono

Okinawa Institute of Science and Technology Graduate University, Membranology Unit, Onna, Okinawa, Japan

Endoplasmic reticulum (ER)-plasma membrane (PM) tethering is crucial for the non-vesicular lipid transport between the ER and PM. However, the PM-associated ER can impede the PM-binding of cytoskeletons and other organelles. It is poorly understood how the competition between the ER and cytoskeletons/organelles on the PM is resolved. Here, we show that, upon septin collar assembly, ER-PM tethering proteins are excluded from the yeast bud sites, and the PM-associated ER is locally detached from the PM. Our results suggest that PM flows by polarized exocytosis extrude PM proteins, including ER-PM tethering proteins, from the bud sites. When the reorganization of the ER-PM tethering was inhibited by exocytosis repression, septin localization was restricted to the PM sites poor in ER-PM tethering proteins. This study demonstrates machinery reconciling ER-septin competition on the PM, providing novel mechanistic insights into the spatial organization of PM-associated organelles and cytoskeletons.

# VAP-MEDIATED MEMBRANE TETHERING MECHANISMS IMPLICATE ER-PM CONTACT FUNCTION IN pH HOMEOSTASIS

Karling Hoh[1,2], Baicong Mu[1,2], Tingyi See[1], Amanda Ng[1,2], Annabel Ng[1], Dan Zhang[1,2]

[1]Temasek Life Sciences Laboratory, Cell Dynamics Group, Singapore, Singapore, [2]National University of Singapore, Department of Biological Sciences, Singapore, Singapore

VAMP-associated proteins (VAPs) are highly conserved endoplasmic reticulum (ER) resident proteins that establish ER contacts with multiple membrane compartments in many eukaryotes. However, VAP-mediated membrane tethering mechanisms remain ambiguous. Here, focusing on fission yeast ER-plasma membrane (PM) contact formation, using systematic interactome analyses and quantitative microscopy, we predict a non-VAP-protein direct binding-based tethering mechanism of VAPs. We further demonstrate that VAP-anionic phospholipids interactions underlie ER-PM association and define the pH-responsive nature of VAP-tethered membrane contacts. Importantly, such conserved interactions with anionic phospholipids are generally defective in amyotrophic lateral sclerosis (ALS)-associated human VAPB mutant. Moreover, we identify a conserved FFAT-like motif locating at the autoinhibitory hotspot of the essential PM proton pump Pma1. This modulatory VAP-Pma1 interaction is crucial for pH homeostasis. We thus propose an ingenious strategy for maintaining intracellular pH by coupling Pma1 modulation with pH-sensory ER-PM contacts via VAP-mediated interactions.

# YEAST LUNAPARK REGULATES FORMATION OF TRANS-ATLASTIN COMPLEXES FOR ER MEMBRANE FUSION

Eunhong Jang, So Young Yoon, Naho Ko, Minseok Kim, Gongmin Na, Youngsoo Jun

Gwangju Institute of Science and Technology, Life Sciences, Gwangju, South Korea

The endoplasmic reticulum (ER) consists of the nuclear envelope and a connected peripheral network of tubules and interspersed sheets. The structure of ER tubules is generated and maintained by various proteins, including reticulons, DP1/Yop1p, atlastins, and lunapark. Reticulons and DP1/Yop1p stabilize the high membrane curvature of ER tubules, and atlastins mediate homotypic membrane fusion between ER tubules; however, the exact role of lunapark remains poorly characterized. Here, using isolated yeast ER microsomes and reconstituted proteoliposomes, we directly examined the function of the yeast lunapark Lnp1p for yeast atlastin Sey1p-mediated ER fusion and found that Lnp1p inhibits Sey1p-driven membrane fusion. Furthermore, by using a newly developed assay for monitoring trans-Sey1p complex assembly, a prerequisite for ER fusion, we found that assembly of trans-Sey1p complexes was increased by deletion of LNP1 and decreased by overexpression of Lnp1p, indicating that Lnp1p inhibits Sey1p-mediated fusion by interfering with assembly of trans-Sey1p complexes.

# THE EXOCYST COMPLEX IS INVOLVED IN AUTOPHAGOSOME BIOGENESIS IN *SACCHAROMYCES CEREVISIAE*

Ruchika Kumari, Sunaina Singh, Ravi Manjithaya

Jawaharlal Nehru Centre for Advanced Scientific Research, Molecular Biology and Genetics Unit, Bangalore, India

Autophagy is an evolutionarily conserved degradative process that is critical for maintaining cellular homeostasis. Autophagy dysfunction has been implicated in several diseases such as neurodegenerative diseases, cancer, lysosomal storage disorders, and intracellular infections. Autophagy involves the *de novo* formation of double-membrane vesicles called autophagosomes that capture cargo to be degraded. The mature autophagosomes then fuse with the lysosomes/vacuoles wherein they are degraded and the by-products are recycled. One of the major breakthroughs was achieved in the field when Prof. Ohsumi's group identified 14 genes that are involved in the pathway using yeast. Following this many laboratories have identified new genes involved in the pathway. The field has extensively grown over the years, however, several open questions remain, such as how does the autophagosome membrane expand? Which organelles serve as membrane sources? What are the genes that are important to mark the autophagosome biogenesis site? Thus, there are still genes that are to be identified to answer these questions. So, to identify such genes, a screen was carried out at the Autophagy Laboratory, JNCASR using yeast. Both essential and non-essential genes were screened using loss-of-function mutants for their involvement in autophagy. One of the hits obtained was the exocyst complex. The exocyst complex is an octameric tethering complex that tethers vesicles to the plasma membrane facilitating its fusion. Further characterization revealed that not all genes of the complex are involved in autophagy suggesting there may be an autophagy-specific exocyst complex. To understand the function of the exocyst complex the loss of function mutants were studied using genetic and fluorescence microscopy approaches. It was revealed that the exocyst plays a role in the early steps of autophagosome biogenesis. Autophagosome biogenesis in yeast takes place at a single perivacuolar site called the phagophore assembly site (PAS). However, further studies are required to understand what is the role of the exocyst complex in autophagosome biogenesis. Currently, biochemical studies are underway to identify interactors of the complex during autophagy.

# CHECKPOINT TARGETS THE UBIQUITIN E3 LIGASE BRL2 TO REGULATE CHROMATIN STRUCTURES IN RESPONSE TO REPLICATION FORK STALLING

Xiaoqin Liu[1], Bo Zhang[1], Yu Hua[1], Xizhou Li[2], Daochun Kong[1]

[1]Peking University, Peking-Tsinghua Center for Life Sciences, The National Laboratory of Protein and Plant Gene Research, College of Life Sciences, Beijing, China, [2]The Naval Military Medical University, Department of Breast and Thyroid Surgery, Changhai Hospital, Shanghai, China

Natural replication barriers frequently cause replication fork stalling. Stalled replication forks are unstable and require sophisticated checkpoint regulation for their stability. Despite intense research over the past two decades, some key questions of underlying checkpoint regulation still remain unanswered. This study identified a new checkpoint target— the ubiquitin E3 ligase Brl2. When replication forks stall, $Cds1^{Chk2}$ phosphorylates Brl2 at five serine residues, which results in sharp reduction of H2BK119ub1 level around stalled replication forks. $Cds1^{Chk2}$ -mediated Brl2 phosphorylation has an epistatic relationship with the sharp reduction of H2BK119ub1. Both *brl2-5D* (the five serine residues mutated to aspartic acid) and *htb-K119R* drastically reduce the sensitivity of $cds1^{Chk2}\Delta$ or $rad3^{ATR}\Delta$ cells to hydroxyurea. Moreover, both *brl2-5D* and *htb-K119R* result in chromatin compaction, which prevents uncoupling of replicative helicase CMG and DNA polymerases, stabilizing stalled replication forks. Thus, this study reveals a novel checkpoint signaling pathway in which nucleosomes are targeted in response to replication fork stalling.

# STRUCTURAL BASIS FOR EUKARYOTIC REPLISOME STALLING AT G-QUADRUPLEXES

Sahil Batra[1], Benjamin Allwein[2], Charanya Kumar[1], Richard Hite[2], Dirk Remus[1]

[1]Memorial Sloan Kettering Cancer Center, Molecular Biology Program, New York, NY, [2]Memorial Sloan Kettering Cancer Center, Structural Biology Program, New York, NY

G-quadruplexes (G4s) are non-canonical DNA secondary structures that are prevalent in G-rich regions of the genome and threaten genome stability by interfering with DNA replication. We have previously found that a single G4 specifically formed on the leading strand can impede the progression of the eukaryotic replicative DNA helicase, CMG (Cdc45-MCM-GINS), resulting in replication fork stalling. Consequently, continued fork progression at G4s is dependent on specialized ancillary DNA helicases that can resolve G4 structures. How G4s impede CMG progression or how ancillary DNA helicases may coordinate G4 resolution with replisome progression is mechanistically not understood. To address these questions, we have reconstituted CMG collisions with a leading strand G4 in the absence or presence of the fork protection complex (FPC, composed of Mrc1 and Csm3-Tof1) with purified budding yeast proteins and characterized the dynamics and structures of CMG stalled at a G4 using biochemical approaches and cryo-electron microscopy (cryo-EM). We were able to determine multiple structural states of the CMG-FPC-G4 complex at < 3 Å resolution, revealing the molecular basis for the G4-induced stalling of eukaryotic replisomes. Moreover, while a G4 embedded in double-stranded DNA (dsDNA) presents a stable physical block to the CMG, we find that DNA unwinding downstream of the G4 can elicit an intrinsic capacity of the CMG to bypass a leading strand G4. Collectively, these data reveal the mechanistic basis for replisome stalling and have important implications for fork restart at G4s.

# POST-REPLICATIVE ACCUMULATION OF RAD51 IS RESOLVED BY RAD54 IN *S. POMBE*

Goki Taniguchi[1], Hiroshi Iwasaki[1,2], Hideo Tsubouchi[1,2]

[1]Tokyo Institute of Technology, School of Life Science and Technology, Yokohama, Japan, [2]Tokyo Institute of Technology, Cell Biology Center, Institute of Innovative Research, Yokohama, Japan

Homologous recombination (HR) contributes to the maintenance of genome integrity, primarily through accurate repair of DNA double-strand breaks. Rad51 is the eukaryotic RecA homolog that assesses the sequence similarity between two DNA molecules and exchanges strands when they are homologous. Multiple Rad51 molecules are recruited to single-stranded DNA exposed near damaged sites, forming a helical nucleoprotein filament structure. Regulating the status of the Rad51 filament is crucial for HR regulation; an excess of Rad51 leads to hyper-oligomerization, which is toxic to the cell. One major factor that controls the Rad51 filament status is Rad54, a SWI/SNF family DNA translocase.

We investigated the role of Rad54 in Rad51 regulation in *S. pombe*. Although HR is defective to a similar level in the *rad51* and *rad54* mutants, the *rad54* mutant grew more slowly than *rad51*. The *rad51 rad54* double mutant showed a similar level of growth speed as the *rad51* single mutant, arguing that the growth difference is attributable to Rad51. Indeed, hyper-aggregation of Rad51 was observed in the *rad54* mutant. Such aggregates typically consist of a focus and a fiber-like structure. Formation of Rad51 aggregates was largely suppressed by the absence of Rad57 and Sfr1, two auxiliary factors of Rad51 promoting its filament formation.

Next, we investigated how Rad51 aggregates are formed in a normally proliferating cell. A transgene expressing Rad51-GFP turned out to be a useful tool for monitoring the in-vivo behavior of Rad51. In wild type, nuclear localization of Rad51 transiently appears as foci around the end of S phase, then disappears as cells enter M phase. In the absence of Rad54, the timing for the appearance of Rad51 is similar to that of wild type, except that the Rad51 signal tends to be more robust and often associated with filament development. The entry into M phase is delayed, or the cell cycle is permanently arrested. A small number of cells enter M phase with persisting Rad51, and those that inherit Rad51 tend to develop much bigger Rad51 aggregates in the next cell cycle.

We propose that DNA damage is produced around the end of S phase in a normally proliferating cell, which requires HR for repair. In the absence of Rad54, recruited Rad51 cannot complete HR, leading to the continuous development of Rad51 aggregates. Such aggregates would be detrimental to healthy cell cycles.

# G4 BINDING AND OLIGOMERIZATION ACTIVITIES OF FISSION YEAST RIF1 PROTEIN ARE REQUIRED FOR LONG-RANGE REGULATION OF ORIGIN FIRING

H. Masai [1], Y. Kanoh [1], D. Kohda[2], T. Sagi[1]

[1]Tokyo Metropolitan Institute of Medical Science, Department of Basic Medical Sciences, Tokyo, Japan, [2]Kyushu University, Medical Institute of Bioregulation, Fukuoka, Japan

Rif1, originally discovered as a telomere binding protein in yeasts, plays a crucial role in determining the firing timings of replication origins. By binding to specific G4 forming sequences on the fission yeast genome, it suppresses the origin firing over 50-100 kb segment. The C-terminal segment of Rif1 contains both G4 binding and oligomerization activities, and are essential for replication timing regulation. We have randomly mutated a Rif1 C-terminal polypeptide and identified mutants specifically defective in G4 binding or oligomerization.

*rif1*I1373M and *rif1*Q1392P mutants in the C-terminal polypeptide (1129-1400aa) exhibit defective oligomerization activity and reduced G4 binding, respectively. Both of these mutants suppress *hsk1-89* mutation (fission yeast counterpart of *cdc7*), indicating that both activities are required for suppressing origin firing. The ChIP seq analyses show that chromatin binding is almost completely lost in Q1392P, suggesting the importance of C-terminal segments-mediated G4 binding. *rif1*I1373M mutant binds to chromatin and suppresses replication at origins near the binding site, but not that at those further away. These results suggest that oligomerization activity of Rif1 C-terminal segment is important for long range suppression of DNA replication.

We also show that overexpression of Rif1 in the wild-type strain results in relocalization of chromatin to nuclear periphery, eventually resulting in mitotic defect and cell death. This indicates that Rif1 regulates not only timely replication of the genome but also proper chromosome localization in nuclei, which is essential for regulated chromosome segregation.

We will also discuss the structural basis of oligomerization and G4 binding of the Rif1 C-terminal segment.

# VISUALIZING HOMOLOGY SEARCH DURING DNA DOUBLE-STRAND BREAK REPAIR

Aoi Makita, Masahiko Harata, Chihiro Horigome*

Tohoku University, Graduate School of Agricultural Science, Sendai, Japan

In budding yeast, DNA double-strand breaks (DSBs) are actively repaired by homologous recombination. Homologous recombination requires a DNA strand with a sequence similar or identical to its own as a template, which is mainly played by sister chromatids after the S phase of the cell cycle. On the other hand, when sister chromatids are not available, it is known that homologous recombination can be performed by searching the entire genome for a donor that can serve as a template. This search for donor by DSB is called homology search, but the details of homology search are still unknown. We have previously shown that DSB sites are relocated to the nuclear periphery (Nagai *et al.*, Science, 2008, Horigome *et al.*, Mol Cell, 2014, Horigome, Bustard, *et al.*, Genes Dev, 2016, Horigome, Unozawa et al., PLOS Genet, 2019), while the machinery for homologous recombination, including Rad52, accumulates at the center of the nucleus (Bystricky *et al.*, Mol Cel Biol, 2009, Dion *et al.*, EMBO report, 2013) . Intriguingly, a previous study showed that a donor sequence on chromosome V is also relocated to the nuclear periphery upon a DSB induction on chromosome III (Oza *et al.*, Genes Dev, 2009).

In this study, we analyzed the dynamics of how a DSB encounters its donor and undergo repair. We exploited a budding yeast strain that can induce a single DSB at the *MAT* locus by galactose-inducible HO endonuclease. In the strain, a *lacO* repeat sequence was inserted close to the artificially inserted donor sequence and the position could be observed as a green fluorescent spot by co-expressing LacI-GFP. This strain further co-expressed a nuclear pore complex fused with GFP, allowing the nuclear periphery to be observed. After induction of cleavage at the *MAT* locus by addition of galactose, the localization of the donor sequence was quantitatively analyzed. In S/G2 phase cells, the donor was relocated to the nuclear periphery and then further moved to the inner side of the nucleus. The sequence of donor movements in the S/G2 phase suggests that DSBs initially make contact with the donor at the nuclear periphery, subsequently migrate to the inner side of the nucleus along with the donor, and are then repaired through homologous recombination. Surprisingly, the donor is relocated to the nuclear periphery and remained there even in G1 phase when homologous recombination is inhibited.

# HISTONE CHAPERONE RTT106 COLLABORATES WITH DNA POLYMERASE ε TO FACILITATE HISTONE PARTITIONING DURING DNA REPLICATION

Zaifeng Hu, Yujie Zhang, Jianxun Feng, Qing Li

Peking University, School of Life Sciences, Beijing, China

Faithful and efficient chromatin replication plays a crucial role in preserving epigenetic information in eukaryotes. Accumulating evidence has shown that histone chaperones and replisome components work in concert during replication to facilitate nucleosome assembly. However, the specific mechanism governing this process remains elusive. In this study, we discovered that the histone chaperone Rtt106 plays a distinct role in both newly synthesized histone deposition and transferring parental histones to the leading strand at replication forks. Intriguingly, we found that Rtt106 interacts with Pol2, the largest subunit of the DNA polymerase ε complex in cells. *In vitro* experiments demonstrate that this interaction is facilitated by histone H3-H4. Notably, we found that this interaction between Rtt106 and Pol2 is independent of Dpb3-Dpb4, which has previously been implicated in parental histone transfer to the leading strands. Interestingly, our domain mapping experiments have revealed that Pol2 possesses a histone-binding domain. Deletion of this domain compromised both the transfer of parental histones and the deposition of new histones. Significantly, disruption of this domain resulted in the loss of heterochromatin silencing. Together, our findings provide a compelling example of the collaborative relationship between histone chaperones and replisome components in facilitating efficient histone partitioning at replication forks. The intricate interplay between these molecular players contributes to epigenetic inheritance in eukaryotes.

# TRANSCRIPTION NEAR ARRESTED DNA REPLICATION FORKS TRIGGERS rDNA COPY NUMBER CHANGES

Mariko Sasaki

National Institute of Genetics, Center for Frontier Research, Shizuoka, Japan

DNA copy number can change as a consequence of structural rearrangements on the chromosome or production and accumulation of extrachromosomal circular DNA. DNA copy number changes can compromise genome stability and cellular integrity and are causes of cancers and numerous other diseases, emphasizing the importance in understanding how DNA copy number changes occur.

The budding yeast ribosomal RNA gene (rDNA) locus offers a unique system to understand mechanisms underlying DNA copy number changes. The budding yeast genome normally contains ~150 rDNA copies, which are tandemly arrayed at a single locus on chr XII. Each rDNA copy contains 35S and 5S rDNAs as well as an origin of DNA replication and a replication fork barrier (RFB) site. Adjacent to the RFB site, there is a regulatory E-pro promoter that transcribes non-coding RNA. After DNA replication is initiated, the replication fork moving in a direction opposite to the 35S rDNA is arrested by a Fob1 protein bound to the RFB site, which leads to formation of a one-ended DNA double-strand break (DSB). DSB end resection, an initiating event for homologous recombination (HR), is normally suppressed, DSBs are repaired by the pathway independent of HR, and rDNA copy number is maintained. When this suppression is relieved, HR is used to repair DSBs but due to the tandemly repeated organization of the rDNA region, HR is prone to deletion or amplification of the rDNA copy on the chromosome as well as production of extrachromosomal rDNA circles.

A histone deacetylase Sir2 suppresses rDNA copy number changes by repressing transcription at the E-pro. Previous studies demonstrate that Sir2-mediated repression at the E-pro facilitates associations of cohesin complexes to the rDNA to suppress rDNA copy number changes. In this study, I demonstrate that Sir2 is also important for suppressing end resection of DSBs formed at the RFB. To examine whether Sir2-mediated repression of the E-pro activity affects DSB repair, I used the strain in which E-pro was replaced by a galactose-inducible *GAL1/10* promoter. When transcription was activated, it enhanced not only DSB formation but also DSB end resection. Moreover, transcription stimulated from E-pro was prematurely terminated by arrested replication forks. These findings led to the model that transcription-replication collision enhances DSB formation and induces DSB end resection and HR-mediated repair that is prone to chromosomal rDNA copy number changes and production of extrachromosomal rDNA circles.

# A SELFISH KILLER GENE IN *SCHIZOSACCHAROMYCES POMBE* DISTORTS TRANSMISSION BY DISRUPTING MITOTIC CHROMOSOME SEGREGATION

Yu Hua[1], Jianxiu Zhang[2], Man-Yun Yang[1], Fan-Yi Zhang[1], Jing-Yi Ren[1], Xiao-Hui Lyu[1], Yan Ding[1], Fang Suo[1], Jun Li[1], Keqiong Ye[2], Li-Lin Du[1]

[1]National Institute of Biological Sciences, Beijing, China, [2]CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Killer meiotic drivers (KMDs) are selfish genetic elements that break Mendel's law by killing non-carrier progeny. A KMD could be mistaken as an essential gene if it kills all deletion progeny of a heterozygous deletion diploid. We find such a gene in *S. pombe* and name it *tdk1* (transmission distorting killer). *tdk1Δ* progeny of *tdk1+* x *tdk1Δ* cross suffer chromosome segregation failures in the first mitosis after spore germination. *tdk1* expresses a single protein product and its killing activity largely relies on a chromatin protein Bdf1. Using cryo-EM, we obtained two different structures of Tdk1, one in complex with Bdf1 and the other in a conformation incompatible with Bdf1 binding. In spores, Tdk1 adopts the Bdf1-binding conformation and forms a supramolecular assembly. Tdk1 expressed during the germination of *tdk1+* spores adopts the non-Bdf1-binding conformation and dissolves the supramolecular assembly, thus preventing any harmful effects. On the other hand, the supramolecular assembly persists after the germination of *tdk1Δ* spores and causes aberrant chromosome adhesion via Bdf1 binding, thereby hindering mitotic chromosome segregation. Our findings reveal how a single-gene-product KMD exploits cellular machinery and gamete-specific cellular environment to exert selfish killing actions.

# CENTROMERE WEAKENING DRIVES EFFICIENT CHROMOSOME FUSION IN FISSION YEAST

Jing Zhang, Siwen Ding, Wenzhu Li, Jingbo Liu, Xiangwei He

Zhejiang University, Life Sciences Institute, Hangzhou, China

Each eukaryotic species has a stable karyotype, which is faithfully maintained through generations. Recent breakthrough in synthetic biology has achieved drastic chromosome fusions in multiple organisms, suggesting that species can tolerate massive changes in karyotype. How chromosome fusion occurs under natural conditions and how it affects species evolution remain to be explored.

Here, we report the finding of a novel function of centromere in maintaining karyotype stability in the fission yeast *S. pombe*. Genetically compromised centromere(s), via reducing the level of CENP-A/Cnp1 incorporation, efficiently induces telomere-telomere fusion, resulting in a stable new karyotype--reducing the haploid chromosome number from three to two. Consequentially, the need in the number of functional centromeres reduces from three to two, compensating for the reduction in CENP-A incorporation. Analysis of the chromosome fusion sites showed that telomere-telomere fusions are carried out by homologous recombination at the sub-telomeric homologous modules. Surprisingly, the patterns of chromosome fusion in cells within a single colony may vary, indicating that chromosome fusion occurs independently and efficiently the daughter cells derived from the same parental cell.

Together, these results support a model that the stability of chromosomal architectures is directly affected by centromere structure/function, and that a possible functional crosstalk exists between centromere and telomere.

# GENOMIC ELEMENTS MEDIATING INTER AND INTRA CHROMOSOMAL INTERACTIONS

Ramveer Choudhary[1], Mohamood Adhil[1,2], Jeffrey Godwin[1], Eugenia Haddad[1], Arti Sharma[1], Michele Giannattasio[1,4], Yathish J Achar[1,3], Marco Foiani[1,4]

[1]IFOM ETS - The AIRC Institute of Molecular Oncology, Genome Integrity Lab, Via Adamello 16, 20139, Milan, Italy, [2]Enhanc3D Genomics Ltd, Cambridge, United Kingdom, [3]Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India, [4]Universita`degli Studi di Milano, Dipartimento di Oncologia ed Emato-Oncologia, Via Festa del Perdono, 7, 20122, Milan, Italy

Our group is focusing on understanding the role of DNA replication and transcription in shaping the topological architecture of chromosomes in Saccharomyces cerevisiae.
We combined Hi-C and ChIA-PET methods to study chromosomal interactions and long-range chromatin interactions associated with specific protein, respectively. We employed chromosomal migration-based assay called Pulse-Field Gel Electrophoresis (PFGE), to analyze topological context of chromosomes as entanglement and migration pattern. By integrating various methods, including bTMP-seq, DRIP-ChIP and H3-ChIP,we characterized the genome-wide topological context of Saccharomyces cerevisiae chromosomes, which involves studying how the DNA strands are spatially organized and interact with each other. We identified regions in the genome that are involved in the formation of negative and positive supercoiling, which are important for the overall stability and structure of chromosomes. We found that transcribed genes generate topological loops and that Top1 and Top2 DNA topoisomerases contribute to their integrity. We also identified genome regions that mediate the formation of intra and inter chromosomal connections. We found that the rDNA locus influences the establishment of chromosomal connections during S phase. These connections are mediated by Type II DNA topoisomerases and resemble catenated structures. We also used electron microscopy to visualize chromatin loops and found evidence for the formation of hemicatenated structures mediating intra chromosomal loops.

# PCNA RECRUITS COHESIN LOADER SCC2/NIPBL TO ENSURE SISTER CHROMATID COHESION

Ivan Psakhye*[1], Ryotaro Kawasumi*[1,2], Takuya Abe[2], Kouji Hirota[2], Dana Branzei[1,3]

[1]IFOM ETS, the AIRC Institute of Molecular Oncology, DNA repair lab, Milan, Italy, [2]Tokyo Metropolitan University, Department of Chemistry, Graduate School of Science, Tokyo, Japan, [3]Istituto di Genetica Molecolare, Consiglio Nazionale delle Ricerche (IGM-CNR), Pavia, Italy

* These authors contributed equally to this work

Sister chromatid cohesion is essential for faithful chromosome segregation and genome duplication during cell division. Failure to establish cohesion during S phase by the ring-shaped multiprotein complex cohesin leads to genomic instability. Replisome-associated proteins are required to generate cohesion by two independent pathways. One mediates conversion of cohesins bound to unreplicated DNA ahead of replication forks into cohesive entities behind them, while the second promotes cohesin de novo loading onto newly-replicated DNAs. The latter process depends on the cohesin loader Scc2/NIPBL and the alternative PCNA loader CTF18-RFC. However, the precise mechanism of de novo cohesin loading during replication is unknown. Here we show that PCNA physically recruits yeast cohesin loader Scc2 via its C-terminal PCNA-interacting protein motif. Binding to PCNA is crucial, as scc2-pip mutant deficient in Scc2-PCNA interaction is defective in cohesion when combined with replisome mutants of the cohesin conversion pathway. Moreover, scc2-pip mutant becomes inviable without its partner Scc4/MAU2 that localizes cohesin loader to centromeres. Importantly, the role of NIPBL recruitment to PCNA for cohesion generation is conserved in vertebrate cells. Our results demonstrate that PCNA, the maestro of replication-linked functions, is also crucially involved in the cohesion establishment through de novo cohesin loading onto replicated DNA.

# 3D GENOME ORGANIZATION IN FISSION YEAST

Ken-ichi Noma[1,2], Osamu Iwasaki[1], Hideki Tanizawa[2], Sanki Tashiro[1]

[1]University of Oregon, Institute of Molecular Biology, Eugene, OR,
[2]Hokkaido University, Institute for Genetic Medicine, Sapporo, Japan

Recent studies indicate that the structural maintenance of chromosomes (SMC) complexes, condensin and cohesin, are involved in genomic contacts and topological chromatin domain organization, although how they establish the 3D genome structure and contribute to nuclear activities remains largely unclear. The condensin and cohesin complexes are highly conserved from simple systems, e.g., yeast cells, to the much more complex human system. Therefore, using 3D genomics methods (in situ Hi-C and ChIA-PET) in addition to genetics and biochemical approaches, we have elucidated the 3D genome-organizing mechanisms in the fission yeast model. We have found that although condensin and cohesin often bind to the same loci, they mediate long- (100 kb – several Mb) and short-range contacts (< 100 kb), respectively, by bridging their binding sites, thereby forming the large and small domains (Kim et al. Nature Genetics 2016). The 300 kb – 1 Mb large domains are typically formed by condensin during mitosis. This mitotic domain organization does not suddenly dissolve but rather diminishes gradually until the next mitosis (Tanizawa et al. Nature Structural & Molecular Biology 2017). Contrarily, the 50 kb small domains formed by cohesin are relatively stable across the cell cycle. The large and small domains are inversely regulated during the cell cycle. Our study predicts that the condensin-mediated large domains serve as chromosomal compaction units. I will discuss how condensin and cohesin are recruited across the genome and how each mediates distinct genome-organizing events (Iwasaki et al. Molecular Cell 205; Noma Annual Review of Genetics 2017).

# SCH$^{9S6K}$ —MEDIATED CONTROL OF DNA REPAIR AND DNA DAMAGE RESPONSE DURING CHRONOLOGICAL AGING

Elisa Ferrari[1], Chiara Lucca[1], Ghadeer Shubassi[1] , Marco Foiani[1,2]

[1] IFOM ETS - The AIRC Institute of Molecularncology, Milan, Italy,[2] Università degli Studi di Milano, Milan, Italy.

Survival to UV-induced DNA lesions relies on nucleotide excision repair (NER) and the DNA damage response (DDR). We studied DDR and NER during chronological and replicative aging. We found that old cells fail to efficiently repair DNA and activate the DDR. We employed pharmacologic, genetic and mechanistic approaches to rescue DDR and repair efficiency during aging. We found that the Torc1, Snf1 and PP2A pathways influence the capability of old cells to repair DNA and to activate the DDR. Moreover, the phosphorylation state of the Sch$^{9S6K}$ kinase can selectively affect NER or DDR efficiency during aging. Altogether our data suggest that DDR and NER are suppressed during aging by metabolic inputs and that metabolic circuits cross-talk with those pathways controlling genome integrity.

# CONDENSIN-MEDIATED CHROMATIN LOOP FORMATION FOR CONTROLLING MITOTIC CHROMOSOME STRUCTURE

Yasutaka Kakui[1,2,3], Christopher Barrington[4], Frank Uhlmann[3]

[1]Waseda university, Waseda Inst for Adv Study, Tokyo, Japan, [2]Waseda university, Grad Sch of Adv Sci and Eng, Tokyo, Japan, [3]The Francis Crick Institute, Chromosome Segregation Laboratory, London, United Kingdom, [4]The Francis Crick Institute, Bioinformatics & Biostatistics Science Technology Platform, London, United Kingdom

Structural maintenance of chromosomes (SMC) complexes are key organisers of chromosome structure throughout the cell cycle. Condensin, a member of SMC complex, embraces chromatin fibre within its ring to facilitate the formation of mitotic chromosomes. Various evidence supports that condensin-dependent chromatin loop formation establishes chromosome structure in mitosis. However, it is still a subject of debate how condensin forms chromatin loops in cells.
We have applied genome-wide chromosome conformation capture (Hi-C) to two yeast species, budding and fission yeasts. Our Hi-C revealed that longer chromatin in fission yeast exhibits larger chromatin loop sizes, while shorter budding yeast chromatin forms smaller loops, indicating that species-specific chromatin loop organisations. Within each yeast species, chromatin loops expand as genomic DNA lengths in chromosomal arms increase. By comparing the pattern of condensin distribution on mitotic chromosomes with the distribution of chromatin loop sizes, we found a relationship between the interval of condensin-binding sites and chromatin loop sizes in both yeasts. Furthermore, our super-resolution microscopy uncovered that the range of chromosome widths correlates with genomic DNA lengths in yeast species. Taken together, our results highlight the cooperative roles of condensin locations and genomic DNA lengths in defining chromatin loop sizes, thereby shaping mitotic chromosomes.

# A FISSION YEAST-BASED PLATFORM FOR SCREENING POTENT HUMAN KINESIN-5/EG5 INHIBITORS

Fara Difka Afdilla[1], Masashi Yukawa[1,2]

[1]Hiroshima University, Graduate School of Integrated Sciences for Life, Higashi-Hiroshima, Japan, [2]Hiroshima University, Hiroshima Research Center for Healthy Aging (HiHA), Higashi-Hiroshima, Japan

Faithful chromosome segregation during cell division is essential for life sustainability and bipolar spindle has the main role to align chromosomes and segregates them into two daughter cells. In human cell, the formation of bipolar spindle is mainly established by the activity of kinesin-5 (Eg5). However, surprisingly, this mitotic-associated protein is abundantly expressed in various human cancer cells and surmised to have correlation with a lower cancer prognosis. Nowadays, human mitotic kinesin motor proteins are expected as the attractive targets in anti-cancer drug discovery, since their inhibitors would have fewer side effects than conventional microtubule inhibitors. Several inhibitors for human kinesin-5 (Eg5), which is essential for mitotic spindle formation, have been developed and subjected to clinical trials. However, they have still not been commercially distributed as anti-cancer drugs due to their poor efficacy and frequent emergence of drug resistant cells. Therefore, development of more effective inhibitors is still needed for clinical application. In order to achieve this efficiently, it is necessary and valuable to establish in vivo assay systems in which to easily monitor the inhibitory effects of kinesin activity. Previously, we reported that the fission yeast cells overproducing Eg5 are useful for screening for its inhibitors [1]. In this study, we show that the fission yeast cells expressing Eg5 as a sole kinesin-5 are sensitive to known-Eg5 inhibitors, thus, are useful to evaluate how these reagents inhibit Eg5 function in vivo. In this presentation, we will introduce how to isolate inhibitory compounds against Eg5 by using fission yeast cells.

References

[1] Hwang, W., Toda, T., Yukawa, M., Biosci. Biotechnol. Biochem., 2022, 86, 254-259.

# RESILIENCE OF TRANSLATIONAL ACTIVITY UNDER ETHANOL STRESS VIA REGENERATION OF SCANNING FACTOR DED1

Ryoko Ando, Shingo Izawa

Department of Applied Biology, Graduate School of Science and Technology, Kyoto Institute of Technology, Kyoto, Japan

Pre-exposure to mild stress often improves cellular tolerance to subsequent severe stress. Severe ethanol stress (10% v/v) causes persistent and pronounced translation repression in *Saccharomyces cerevisiae*. However, whether pre-exposure to mild stress can mitigate translation repression in yeast cells under severe ethanol stress remains unclear. We found that the translational activity of yeast cells pretreated with 6% (v/v) ethanol was initially significantly repressed under subsequent 10% ethanol but was then gradually restored even under severe ethanol stress. We also found that 10% ethanol caused aggregation of Ded1, which plays a key role in translation initiation as a DEAD-box RNA helicase. Pretreatment with 6% ethanol led to the gradual disaggregation of Ded1 under subsequent 10% ethanol treatment in wild-type cells but not in *fes1*Δ*hsp104*Δ cells, which are deficient in Hsp104 with significantly reduced capacity for Hsp70. Hsp104 and Hsp70 are key components of the bi-chaperone system that play a role in yeast protein quality control. *fes1*Δ*hsp104*Δ cells could also not restore translational activity under 10% ethanol even after pretreatment with 6% ethanol. These results indicate that regeneration of Ded1 through the bi-chaperone system leads to gradual restoration of translational activity under continuous severe stress. Aggregation and regeneration of Ded1 might be one of the key mechanisms for translation regulation in yeast cells under ethanol stress. This study provides new insights into the acquired tolerance of yeast cells to severe ethanol stress and the resilience of translational activity. Notably, we recently found that 10% ethanol at 15°C had a less inhibitory effect on translation with little aggregation of Ded1 than at 28°C. We are further investigating the effect of temperature during ethanol stress treatment.

# PHYSIOLOGICAL ANALYSIS OF *S. CEREVISIAE* CELLS EXPRESSING CRITICAL AMOUNT OF EXCESS PROTEINS

Yuri Fujita[1], Hisao Moriya[2]

[1]Okayama University, Environmental and Life Science, Okayama, Japan,
[2]Okayama University, Environmental Life Natural Science and Technology, Okayama, Japan

Studies have been conducted with overexpression of excess proteins in order to determine the principle on which the amount of each protein in the cell is optimized. In a study using *E. coli*, it has been observed that growth inhibition occurs when excess proteins are expressed up to about 15% of the total proteins, and estimated that growth ceases completely at about 37% (Bruggeman *et al*., *FEMS Microbiol. Reviews* 2020). We also found that overexpression of 15% of excess proteins (green fluorescent protein, glycolytic enzymes, etc.) inhibited growth in the budding yeast *S. cerevisiae* (Eguchi *et al*., *eLife* 2018).

In this study, we further explored the degree to which budding yeast tolerates the expression of excess proteins. We found low toxicity proteins that yeast maintained growth while expressing as much as 40-50% of total proteins, including a mutated fluorescent protein (mox-YG) and glycolytic enzymes. We then analyzed the transcriptome, proteome, fluorescence microscopy, and electron microscopy of cells with critical expression of mox-YG. We observed a general decrease in expression of proteins other than mox-YG, an amino acid starvation response, decreased expression of ribosomal proteins, and a shrinking nucleolus. On the other hand, mitochondrial development was observed, suggesting that critical expression of excess proteins causes conversion to efficient metabolism.

# TOP2-MEDIATED CHROMATIN STRUCTURAL AND TOPOLOGICAL DYNAMICS IN G2/M

Eugenia Haddad[1], Mohamood Adhil[1,2], Ramveer Choudhary[1], Jeffrey Godwin[1], Yathish Achar[1,3], Marco Foiani[1,4]

[1] FIRC Institute of Molecular Oncology, Genome Integrity, Milan, Italy, [2]Enhanc3D Genomics Ltd Rheinische Friedrich-Wilhelms-Universitat Bonn, Enhanced Genomics, Cambridge, United Kingdom, [3]Centre for DNA Fingerprinting and Diagnostics, Genome Architecture, Hyderabad, India, [4]University of Milan, Molecular Biology, Milan, Italy

DNA topoisomerases, Top1 and Top2, together with the high mobility group protein Hmo1, assist DNA replication and transcription to avoid topological stress and ensure genome stability. Hmo1 and Top2 contribute to the architectural state of transcription by stabilizing cruciform structures and protecting negative supercoils that are formed in gene boundaries in S phase. While the topological state of the genome has been characterized in G1 and S phases of the cell cycle, a clear picture of genome topology in G2/M is still missing.
Here we investigated the DNA supercoiling state of the genome in G2/M arrested cells of Saccharomyces cerevisiae using biotinylated 4,5,8-trimethylpsoralen (bTMP) chromatin immunoprecipitation (CHIP) technique. We found that, differently from S phase cells, Top2 counteracts the accumulation of negative supercoiling and RNA-DNA hybrids at transcribed genes. Further investigation will be continued to determine whether this accumulation of negative supercoiling is due to condensin and/or Hmo1.
A comparison between the genome topological state in G1, S and G2/M will be shown.

# SYNONYMOUS tRNA GENES ALL THE SAME OR NOT?

Sachiko Hayashi[1], Masaya Matsui[2], Ayano Ikeda[2], Moeka Taniwaki[1], Tohru Yoshihisa[1]

[1]Univ. of Hyogo, Grad. Sch. of Sci., Ako-gun, Japan, [2]Univ. of Hyogo, Facul. of Sci., Ako-gun, Japan

*Saccharomyces cerevisiae* has 275 tRNA genes (tDNAs) including 61 intron-containing tDNAs, to produce 41 tRNA species defined by anticodon. The copy number of synonymous genes for an isoacceptor tRNA, a primary determinant for tRNA availability, has been thought to be strongly linked to the codon usage in various organisms. Nevertheless, our mutant analysis of $tRNA^{Trp}$ synonymous genes to reduce their copy number from 6 (wild type) to 1 sequentially indicates that reduction of the copy number to 2 does not have an apparent impact on cell growth. Further, $tDNA^{Trp}$ quintuple-deletion mutants possessing single $tDNA^{Trp}$ at the *J*, *M*, or *P* locus exhibit more severe growth defects than those possessing single $tDNA^{Trp}$ at the *G1*, *G2*, or *K*. Because the quadruple deletion strains with all possible combinations expressed nearly one-third of mature $tRNA^{Trp}_{CCA}$ in the wild type, all the 6 $tDNA^{Trp}$ loci are supposed to contribute to $tRNA^{Trp}$ production almost equally. Thus, growth variations among the quintuple deletion strains may not simply reflect differences in $tRNA^{Trp}_{CCA}$ shortage. We also observed opposite cases. We examined whether individual transcription among synonymous tDNAs differs, especially during stress responses. qRT-PCR analyses were performed using a specific primer set against an intron in a specific $tRNA^{Leu}_{CAA}$ or $tRNA^{Phe}_{GAA}$ gene to distinguish its primary transcript from those derived from the other synonymous tDNA loci. Under fermentable and respiratory conditions, one of the synonymous genes of $tRNA^{Phe}_{GAA}$ and $tRNA^{Leu}_{CAA}$, namely *tL(CAA)D* and *tF(GAA)D*, respectively, was expressed less than a theoretical amount assumed from their copy numbers. The amount of the $tRNA^{Phe}_{GAA}$ primary transcript responded differently between *tF(CAA)D* and other $tDNA^{Phe}$ loci upon heat shock stress but similarly upon oxidative stress. In these cases, transcription of at least one synonymous gene for $tRNA^{Phe}_{GAA}$ and $tRNA^{Leu}_{CAA}$ behaves differently from the others. In summary, the budding yeast retains redundancy of synonymous tDNAs for some isoacceptor tRNAs with an equal contribution for tRNA supply, while some synonymous genes for certain isoacceptor tRNAs seem to be regulated differently from the other synonymous genes at transcription.

# MOLECULAR CONNECTION OF LIPID BIOGENESIS AND MITOCHONDRIAL PROTEIN IMPORT

Sandra Heinen, Thomas Becker

University of Bonn, Institute of Biochemistry and Molecular Biology, Bonn, Germany

Mitochondria are important organelles that generate the energy for various biochemical processes in a cell. The majority of the proteins that are needed inside mitochondria to fulfill functions like ATP production, lipid and heme biosynthesis are synthesized by cytosolic ribosomes and are imported into mitochondria afterwards. The protein Ayr1 is localized at the mitochondrial outer membrane, the ER membrane and lipid droplets. It plays a dual role during lipid synthesis. On the one hand it drives the synthesis of phosphatidic acid in a NADPH-dependent manner. On the other hand, the triacylglycerol lipase Ayr1 is involved in the mobilization of triacylglycerols and sterol esters from lipid droplets. However, the actual function of Ayr1 at mitochondria remains unclear. Here we report that Ayr1 is a novel interaction partner of the mitochondrial import complex (MIM complex). Furthermore, we could show that overexpression of mitochondrial Ayr1 strongly affects mitochondrial functions and supports the association of lipid droplets with mitochondria. Altogether, the interaction of the MIM complex with Ayr1 represents a molecular connection between protein import and lipid metabolism.

# VAP-MEDIATED MEMBRANE TETHERING MECHANISMS IMPLICATE ER-PM CONTACT FUNCTION IN pH HOMEOSTASIS

Kar Ling Hoh[1,2], Baicong Mu[1,2], Tingyi See[1], Amanda Yunn Ee Ng[1,2], Annabel Qi En Ng[1], Dan Zhang[1,2]

[1]Temasek Life Sciences Laboratory, Singapore, [2]National University of Singapore, Department of Biological Sciences, Singapore

VAMP-associated proteins (VAPs) are highly conserved endoplasmic reticulum (ER) resident proteins that establish ER contacts with multiple membrane compartments in many eukaryotes. However, VAP-mediated membrane tethering mechanisms remain ambiguous. Here, focusing on fission yeast ER-plasma membrane (PM) contact formation, using systematic interactome analyses and quantitative microscopy, we predict a non-VAP-protein direct binding-based tethering mechanism of VAPs. We further demonstrate that VAP-anionic phospholipids interactions underlie ER-PM association and define the pH-responsive nature of VAP-tethered membrane contacts. Importantly, such conserved interactions with anionic phospholipids are generally defective in amyotrophic lateral sclerosis (ALS)-associated human VAPB mutant. Moreover, we identify a conserved FFAT-like motif locating at the autoinhibitory hotspot of the essential PM proton pump Pma1. This modulatory VAP-Pma1 interaction is crucial for pH homeostasis. We thus propose an ingenious strategy for maintaining intracellular pH by coupling Pma1 modulation with pH-sensory ER-PM contacts via VAP-mediated interactions.

# EXPLORING THE GENETIC LANDSCAPE OF YEAST METABOLISM

Ira Horecka, Michael Costanzo, Charles Boone, Hannes Röst

University of Toronto, Department of Molecular Genetics, Toronto, Canada

The yeast metabolic network precisely controls metabolic flux under various environmental conditions. This network is comprised of metabolic pathways, with each pathway composed of enzymes performing specific metabolic functions. Exploring this complexity often requires genetic disruption of the system. However, the network's resilience to shifting environmental conditions presents a challenge in revealing subtle metabolic relationships when studying individual genes. Therefore, we hypothesize that identifying significant sets of genetic interactions between metabolic pathways will reveal complementary and redundant mechanisms at a higher level, improving our understanding of the metabolic network.

In this study, we developed a rigorous statistical pipeline to comprehensively identify significant sets of positive and negative genetic interactions between 475 metabolic pathways. We discovered 13,082 such pathway-pathway interactions (FDR < 0.05), validating established connections and revealing novel metabolic relationships. Additionally, to investigate how genes contribute to metabolic signaling, we expanded our statistical analysis to include the yeast genome. This revealed an additional 15,518 significant gene-pathway interactions and predicted metabolic functions for previously uncharacterized genes.

Finally, we found that pathways involved in larger metabolic functions, such as cellular respiration, tend to share similar profiles of genetic interactions. We exploited this property to construct a global metabolic network, grouping pathways with similar interaction patterns together. This network illustrates genetic relations between diverse metabolic processes and maps their functional organization in the cell.

In summary, we present a robust statistical pipeline to investigate the genetic landscape of yeast metabolism at the pathway level, thus enhancing existing gene-gene data with prior knowledge. The modularity of our pipeline promises the integration of diverse high-throughput datasets, thereby enriching our metabolic network. We anticipate these predictions to guide future experiments aimed at exploring novel metabolic relationships in the cell.

# EXPLORING THE MOLECULAR MECHANISM OF ACTIN-DEPENDENT MITOTIC NUCLEAR POSITIONING

Woosang Hwang[1], Masashi Yukawa[1,2]

[1]Hiroshima University, Graduate School of Integrated Sciences for Life, Hiroshima, Japan, [2]Hiroshima University, Hiroshima Research Center for Healthy Aging (HiHA), Hiroshima, Japan

All eukaryotic cells activate a spindle assembly checkpoint (SAC) if they have abnormal spindle formation or improper spindle-kinetochore attachment: a mechanism by which to prevent precocious chromosome segregation. However, mitotic arrest induced by the SAC in general does not last forever; instead, cells eventually exit mitosis abnormally without chromosome segregation. For instance, in fission yeast, mitotically arrested cells undergo septation in the absence of chromosome segregation, so that the septum physically divides the chromosome into two parts, leading to inviable daughter cells (referred to as “cut”). We previously reported unexpected observations that the nucleus is displaced from the cell center by actin filaments in several mitotic mutants. This actin-dependent mitotic nuclear displacement may provide an advantage to mitotically arrested cells for survival, because survivors in mitotic mutants can resume cell division as diploid progenies at the permissive temperature. In this study, we have investigated the precise timing of nuclear displacement during mitosis and found that mitotic nuclear displacement often occurs during maturation of a contractile actomyosin ring (CAR) at putative septation sites. We also noticed that the CAR is formed at a place slightly away from the cell center. Intriguingly, the nucleus tends to move towards the pole opposite to that to which the CAR is displaced, thereby preventing cut. To clarify underlying mechanisms, we are currently examining the dynamics of actin filaments just before nuclear displacement. In this presentation, we will show our recent results and discuss how mitotically arrested cells relocate the nucleus during mitosis.

# MEMBRANE CONTACT SITES REGULATE VACUOLAR FISSION VIA SPHINGOLIPID METABOLISM

Atsuko Ikeda[1], Kazuki Hanaoka[1], Kensuke Nishikawa[1], Philipp Schlarmann[1], Sayumi Yamashita[2], Aya Nakaji[2], Sotaro Fujii[1], Kouichi Funato[1,2]

[1]Hiroshima University, Graduate School of Integrated Sciences for Life, Kagamiyama 1-4-4, Higashi-Hiroshima, Japan, [2]Hiroshima University, School of Applied Biological Science, Kagamiyama 1-4-4, Higashi-Hiroshima, Japan

Membrane contact sites (MCSs) are junctures that perform important roles including coordinating lipid metabolism. Previous studies have indicated that vacuolar fission/fusion processes are coupled with modifications in the membrane lipid composition. However, it has been still unclear whether MCS-mediated lipid metabolism controls the vacuolar morphology. Here we report that deletion of tricalbins (Tcb1, Tcb2, Tcb3), tethering proteins at endoplasmic reticulum (ER)-plasma membrane (PM) and ER-Golgi contact sites, alters fusion/fission dynamics and causes vacuolar fragmentation in the yeast Saccharomyces cerevisiae. In addition, we show that the sphingolipid precursor phytosphingosine accumulates in tricalbin-deleted cells, triggering the vacuolar division. Detachment of the nucleus vacuole junction (NVJ), an important contact site between the vacuole and the perinuclear ER, restored vacuolar morphology in both cells subjected to high exogenous phytosphingosine and Tcb3-deleted cells, supporting that phytosphingosine transport across the NVJ induces vacuole division. Thus, our results suggest that vacuolar morphology is maintained by MCSs through the metabolism of sphingolipids.

# RESILIENCE TO SEVERE ETHANOL STRESS IN THE YEAST UBIQUITIN-PROTEASOME SYSTEM

Vo Thi Anh Nguyet, Ryoko Ando, Noboru Furutani, Shingo Izawa

Kyoto Institute of Technology, Applied Biology, Kyoto, Japan

Although the budding yeast, *Saccharomyces cerevisiae*, produces ethanol via alcoholic fermentation, high-concentration ethanol is harmful to yeast cells. Severe ethanol stress (> 10% v/v) has adverse effects on protein synthesis and protein quality. However, its effect on proteolysis remains largely unknown. We examined the effects of ethanol on yeast proteasomal proteolysis via the cycloheximide-chase analysis of short-lived proteins (Rim101 and Gic2). We also assayed protein degradation in the auxin-inducible degron system (Paf1-AID*-6FLAG) and the ubiquitin-independent degradation of Spe1 under ethanol stress. We found that severe ethanol stress strongly inhibited the degradation of proteasomal target proteins in an ethanol-concentration dependent manner. Therefore, proteasomal proteolysis resumed rapidly once the ethanol was removed. Furthermore, yeast cells pretreated with mild ethanol stress (6% v/v) showed proteasomal proteolysis even with 10% ethanol, indicating that yeast cells acquired resistance to proteasome inhibition caused by severe ethanol stress. However, yeast cells failed to acquire sufficient resistance to severe ethanol stress when new protein synthesis was blocked during pretreatment, or when Rpn4 was lost. Interestingly, in the low temperature range (around 15°C), proteasomal proteolysis was not inhibited in yeast cells under severe ethanol stress, and sufficient proteasomal activity was maintained. These results indicated that yeast proteasome activity under severe ethanol stress was substantially affected by temperature. Our findings provide novel insights into the effects of ethanol and low temperature on yeast physiology.

# THE ROLE OF FATTY ACID-BINDING PROTEIN 4 IN THE DEVELOPMENT OF OBESITY-RELATED BENIGN PROSTATIC HYPERPLASIA.

Mengxue Jiang[1,2]

[1] Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [2]State Key Laboratory of Pharmaceutical Biotechnology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

**Introduction**: Benign prostatic hyperplasia (BPH) is characterized by overgrowth of prostatic epithelial cells and stromal cells, which lead to enlargement of prostate and lower urinary tract symptoms. In addition to aging, emerging evidence suggests that obesity is a leading pathogenic factor of BPH while the underlying mechanism remains obscure. Fatty acid-binding protein 4 (FABP4) is an adipokine that regulates lipid transportation and metabolism. Elevated circulating FABP4 is a well-established risk factor for obesity-related metabolic diseases and has been shown to promote prostate cancer development under obese condition. Here, we investigate whether elevated circulating FABP4 stimulates prostatic cell proliferation, thus potentiating obesity related BPH.
**Method**: Six-week-old FABP4 knockout (FABP4 KO) mice and wildtype (WT) mice were subjected to either high fat diet (HFD, 60% calorie from fat) or standard chow diet feeding for three months. Mouse body weight and fat mass percentage were monitored biweekly. After euthanasian, the size and weight of prostate lobes were recorded, and the histological changes were determined by H&E staining. The expression of cell proliferating markers in mouse prostate was examined by western blotting. The underlying mechanism of FABP4 in promoting prostatic cell proliferation was further investigated by treating human benign prostatic hyperplasia cells (BPH-1) with human recombinant FABP4 protein (hFABP4).
**Result**: Circulating FABP4 was significantly elevated in WT mice upon HFD feeding, while the expression of FABP4 in the mouse prostate was undetectable. HFD-induced prostate enlargement was significantly attenuated in FABP4 KO mice when compared to the WT mice. Histologically, HFD-induced prostate epithelial cell and stromal cell overgrowth were attenuated in FABP4 KO mice. Furthermore, the upregulation of proliferating cell nuclear antigen (PCNA) in WT mouse prostate upon HFD feeding was suppressed in that of FABP4 KO mice. In parallel, treatment of hFABP4 significantly induced the expression of cell proliferation marker (PCNA and Ki67) in BPH-1 cells.
**Conclusion**: The results implicated that elevated circulating FABP4 under obese condition stimulates prostate cell proliferation, thus potentiating the development of BPH. Targeting FABP4 may be a promising strategy for treating obesity related BPH.

# INVESTIGATION OF MODIFIED SIDE CHAIN-COENZYME Q MOLECULES IN BLACK YEAST-LIKE FUNGI AND OTHER FUNGI

Jomkwan Jumpathong[1], Ikuhisa Nishida[2], Tomohiro Kaino[3], Makoto Kawamukai[3]

[1]Tottori University, Bioresource Science, Tottori, Japan, [2]Niigata University, Sakeology Center, Niigata, Japan, [3]Shimane University, Life and Environmental Science, Matsue, Japan

Coenzyme Q (CoQ) is an essential electron transporter in the electron transport system in both prokaryotes and eukaryotes. The CoQ profile is linked to the indication of taxonomic classification in all organisms. *Aureobasidium* species, which are yeast-like fungi (fungi that develop in yeast form in the early stages of life), are frequently found to produce modified CoQ in the tail by saturation of certain isoprene units - causing a change in its molecular structure. *Aureobasidium* species reportedly produce $CoQ_{10}(H_2)$ as a major species, but the species that produce $CoQ_{10}(H_4)$ has never been found before. In this experiment, we have newly identified a species belonging to *Aureobasidium* from nature, termed A37, that produce $CoQ_{10}(H_4)$ as a major CoQ species from the stationary phase of growth. A37 has interesting morphological characteristics with cells having excessive melanization. In this experiment, the morphology is observed in PDB (dextrose containing medium) and YPD (rich medium), and under microscopic observation. Interestingly, we found that A37 prefers to retain its yeast form when the nutrient is abundant. To more clearly investigate its CoQ profile, *A. pullulans* Type strain (EXF-150) and *Sydowia polyspora* NBRB 30562, the latter which retains its yeast form only in the very early stage of growth, were observed in parallel. The observation was also done comparing to other filamentous fungi *Aspergillus oryzae* and *Aspergillus terreus.*

Our mass spectral based analysis showed that only A37 produced $CoQ_{10}(H_4)$ as a major CoQ species, and no other yeast-like fungi did. We also observed that A37 has the most various saturated and unsaturated (regular) CoQ species identified amongst all. The presence of both regular and saturated $CoQ_{10}$ indicates that the saturation occurs after the benzoquinone head and the isoprenoid tail are successfully condensed.

The HPLC analysis and the microscopic observation showed that when A37 is grown with the limited nutrients (dextrose as carbon source), it produces $CoQ_{10}(H_2)$ as a major CoQ species with the highest ratio of $CoQ_{10}(H_4)/CoQ_{10}(H_2)$ at 0.4 when cultivated for 18 days at 30 °C (and 0.28 at 25 °C). In contrast, when grown in YPD, the ratio reached approximately 1.5 when cultivated for 18 days.

This result indicated A37 has a long-life cycle and the cells keep dividing even as longer than 20 days in YPD while retaining a yeast form. We may be able to conclude that, when grown in abundant nutrients, the cells choose to retain its yeast form in order to grow more freely.

# OLIGOMERIZATION OF RIF1 IS ESSENTIAL FOR ITS ABILITY TO SUPPRESSES REPLICATION OVER A LONG DISTANCE IN *S. POMBE*.

Yutaka Kanoh, Kaho Takasawa, Seiji Matsumoto, Hisao Masai

Tokyo Metropolitan Institute of Medical Science, Department of Basic Medical Sciences, Tokyo, Japan, Japan

Rif1, originally discovered as a telomere length regulator, binds to chromatin via G-quadruplex (G4) and inhibits replication origin firing in 50~100 kb segment surrounding the binding site in *S. pombe*. This long distance suppression is likely to involve chromatin higher-order structure generated by Rif1 binding. Previously we reported Rif1 N- and C-terminal segment bind to G4 with a high affinity and the latter form oligomers [1]. Overexpression of Rif1 in fission yeast cells induced cell death by causing chromatin to relocate to nuclear periphery [2]. However, contribution of Rif1 oligomerization on replication timing regulation has not been addressed. Here, we have mutated the C-terminal segment of Rif1 to obtain mutants which are deficient in oligomerization. We randomly mutated Rif1C-terminal polypeptide (1129-1400aa) and identified *rif1Q1392P* and *rif1I1373M* mutations. Purified *Q1392P* or *I1373M* in polypeptide (1129-1400aa) showed compromised G4 binding or oligomerization, respectively. The mutations were reintroduced in the endogenous Rif1 loci, and showed that both could restore the viability of *hsk1-89* lethality (cdc7 counterpart), indicative of loss of Rif1 function. Indeed, the initiations of DNA replication at late replication origins were deregulated in these mutants. Interestingly, *I1373M* mutant could bind to chromatin and suppressed the replication at origins present near (~20 kb) of the binding site, but did not suppress replication at those further away. These results suggested that oligomerization activity of Rif1 C-terminal segment is important for long range suppression of DNA replication.

References

1. Kobayashi S. *et al*. *Mol Cell Biol*. 2019 Feb 4;39(4):e00364-18.
2. Kanoh Y. *et al*. *Life Sci Alliance*. 2023 Feb 7;6(4):e202201603.

# PHYSICOCHEMICAL PROPERTIES OF THE VACUOLAR MEMBRANE AND CELLULAR FACTORS DETERMINE FORMATION OF VACUOLAR INVAGINATIONS

Yoko Kimura[1,3], Takuma Tsuji[2], Yosuke Shimizu[1], Masafumi Kimura[1], Toyoshi Fujimoto[2], Miyuki Higuchi[3]

[1]Shizuoka University, Grad. Sch. Integ. Sci.& Tech, Shizuoka, Japan, [2]Juntendo University, Grad. Sch. Med., Tokyo, Japan, [3]Shizuoka University, Grad. Sch. Integ. Sci.& Tech, Shizuoka, Japan

Vacuoles change their morphology in response to stress. In yeast exposed to chronically high temperatures, vacuolar membranes get deformed and invaginations are formed. We show that phase-separation of vacuolar membrane occurs after heat stress leading to the formation of the invagination. In addition, Hfl1, a vacuolar membrane-localized Atg8-binding protein, suppresses the excess vacuolar invaginations after heat stress. At that time, Hfl1 forms foci at the neck of the invaginations in wild-type cells, whereas it is efficiently degraded in the vacuole in the *atg8*Δ mutant. Genetic analysis showed that the endosomal sorting complex required for transport (ESCRT) machinery is necessary to form the invaginations irrespective of Atg8 or Hfl1. In contrast, a combined mutation with the vacuole BAR domain protein Ivy1 leads to vacuoles in *hfl1Δ ivy1Δ* and *atg8Δivy1Δ* mutants having constitutively invaginated structures; moreover, these mutants show stress-sensitive phenotypes. Our findings suggest that vacuolar invaginations result from the combination of changes in the physiochemical properties of the vacuolar membrane and other cellular factors.

# THE RESPONSE TO ROS PRODUCED BY METALS IN VARIOUS SACCHAROMYCES YEASTS

Shunji Kiyoda[1], Norio Ito[1], Yoshiharu Tanaka[1], Ryoko Asada[1], Masakazu Furuta[1], Masao Kishida[2]

[1]Graduate School of Engineering, Osaka Metropolitan University, Quantum and Radiation Engineering, Sakai, Japan, [2]Graduate School of Agriculture, Osaka Metropolitan University, Applied Biological Chemistry, Sakai, Japan

Manganese (Mn), playing an essential role to the cellular activities with the micromolar scale, plays a toxic material occurring the oxidative damage at more than 1 milimolar in *Saccharomyces cerevisiae* BY4741. *S. cerevisiae* IM3, isolated from BY4741 as a tolerant mutant to higher concentration of Mn, has four-to-five-fold Mn absorption ability under the cultivation with Mn containing media. IM3 shows the less resistant to γ-ray irradiation than BY4741. In BY4741, Mn supplementation into nutrient medium showed significant resistant to reactive oxygen species (ROS) caused by some sources, such as γ-ray irradiation, menadione, and hydrogen peroxide while the ROS resistance is decreased in IM3. Next, we examined the effect of zinc (Zn), causing an oxidative toxicity, on BY4741 and IM3. Since IM3 was more sensitive than BY4741, the ROS production is suggested to be correlating with the metal absorption in *Saccharomyces* yeast. We also examined the absorption of metals and the sensitivity of the oxidative stress using the γ-ray irradiation in the other laboratory strains, NA87-11A and DKD-5DH, and the brewing strains, EC1118, AKU4102, IFO 2044 (Oriental yeast), and Association yeast No. 7 from Brewing Society of Japan, in the *Saccharomyces* yeast.
Among the latter six strains, the Mn absorption of five strains except for Association yeast No. 7 were similar to that of BY4741, however, the Mn absorption of Association yeast No. 7 was more than IM3. Similar results were shown in the Zn absorption. The response to oxidative stress by the γ-ray irradiation of Association yeast No. 7 absorbed Zn was similar to that of BY4741. Moreover, we examine the effect of copper (Cu), similar effect to Zn, on all strains. Cu absorption within the AKU4102 strain increase the resistance to γ-rays while NA87-11A strain was more sensitive to γ-rays than the AKU4102 strain in the absence of Cu. In the case of Zn, there was the similar change in resistance to γ-ray irradiation compared to the no-additive medium. These results suggest that the absorption of the toxic metals with high concentration, like Mn, Zn and Cu, is decreased the resistance to oxidative stresses, while those also suggest that the difference of the absorbing ability between yeast strains is affecting the resistant level to the oxidative stress. We are currently examining ROS amount and activities of antioxidation enzymes in the yeast cells in relation with Mn, Cu or Zn absorption.

# CDK11 INHIBITION DISRUPTS DNA DAMAGE RESPONSE AND ENHANCES ANTI-TUMOR IMMUNITY IN HEPATOCELLULAR CARCINOMA

Zhijian Kuang, Gengchao Wang

State Key Laboratory of Liver Research, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Department of Pathology, Hong Kong, China

Cyclin-dependent kinases (CDKs) belong to the serine/threonine kinases family that play pivotal roles in cell cycle control and transcriptional regulation. Targeting CDKs is emerging as a promising strategy for cancer therapy. In this study, we employed a genome-wide CRISPR/Cas9 library screening to identify CDK11 as an essential gene for hepatocellular carcinoma (HCC) cell proliferation. Pathologically, high expression of CDK11 indicates poor survival in HCC patients, suggesting the clinical significance of CDK11. CDK11 regulates gene transcription elongation by phosphorylating the carboxy-terminal domain of RNA polymerase II. Importantly, we found that inhibition of CDK11 with a small molecular inhibitor (OTS964) or via genetic perturbation hindered the DNA damage response through the transcriptional suppression of multiple DNA repair genes in HCC cells. In conclusion, our research illuminated the function and mechanism of CDK11 in the regulation of DNA repair genes, underscoring its therapeutic potential in HCC treatment.

# BIOSYNTHESIS OF CUCURBITACIN B PRECURSORS IN *SACCHAROMYCES CEREVISIAE*

Yi-Chen Lo, Pei-Chen Su, Yuan-Rui Deng, Hsin-Jo Chang

National Taiwan University, Institute of Food Science and Technology, Taipei, Taiwan

Cucurbitacins are a class of bitter, highly oxygenated tetracyclic triterpenes generated by the plant family Cucurbitaceae. These compounds consist in a variety of forms, including cucurbitacin B with pharmacological properties, such as anti-bacterial and anti-tumor activities. Due to the complexity of the structure and low contents of this compound in plants, it is difficult to be synthesized chemically or extracted by solvents. Therefore, biosynthesis using yeast, Saccharomyces cerevisiae, can be an alternative method to obtain cucurbitacin B. We have successfully engineered cells producing cucurbitadienol, the precursor of cucurbitacin B. However, further hydroxylation on specific carbons and acyl transfer modification of the cucurbitadienol are required for the synthesis of cucurbitacin B. In addition, CYPs were co-expressed with cytochrome P450s reductase to produce 11-carbonylcucurbitadienol and 11-carbonyl-20β-hydroxycucurbitadienol. However, to enable the synthesis of cucurbitacin B in the future, a deeper understanding of the genes related to the final modification of cucurbitacin B precursors will necessitate further characterization.

# CREATING A SINGLE-CHROMOSOME YEAST CELL WITHOUT SPLICEOSOMAL INTRONS

Xin Man, Wen-Ting Zhang, Jin-Qiu Zhou

Chinese Academy of Sciences, Center for Excellence in Molecular Cell Science, Shanghai, China

There are a large number of sequences in the genome of eukaryotes that are not transcribed into functional RNA or translated into proteins, including intergenic sequences and some intragenic introns. In this study, we aim to construct a yeast cell that contains no spliceosomal introns. We used the single-chromosome Saccharomyces cerevisiae yeast strain SY14 as the starting strain. After parallel intron-knockout in SY14α and SY14a cells, chromosomal site-specific exchange, and meiosis-mediated integration of intron-loss genes into one chromosome, we currently obtained the strain SYE6a, in which all of the 64 spliceosomal introns in 57 essential genes were deleted. Compared with SY14, SYE6a exhibited a slightly slower but stable growth rate on rich medium. In the future, we will delete 237 intron in 227 non-essential genes. The completion of this work likely represents a significant progress in the construction of "smallest-genome" eukaryotic cell.

# INSIGHTS INTO THE ROLE OF THE CONSERVED GTPASE DOMAIN RESIDUES IN THE STRUCTURE-FUNCTION RELATIONSHIP OF YEAST DNM1

Riddhi Banerjee[1], Agradeep Mukherjee[1], Shikha Sharma[2], Ehesan Ehesan Ali[2], Shirisha Nagotu[1]

[1]Indian Institute of Technology Guwahati, Biosciences and Bioengineering, Guwahati, India, [2]Institute of Nano Science and Technology, Mohali, India

Mitochondrial division is an extremely regulated process. The master-regulator of this process is the multi-domain, conserved protein, called Dnm1 in yeast. In this study, we systematically analyzed two residues reported to be putatively phosphorylated in the GTPase domain of the protein. These residues lie in the G2 and G5 motifs of the GTPase domain. Both the residues are important for the function of the protein as evident from in vivo and in vitro analysis of the non-phosphorylatable and phosphomimetic variants. Interestingly, mutant variants showed differences with respect to Dnm1 puncta dynamics and localization in vivo albeit both were non-functional as assessed by mitochondrial morphology and GTPase activity. Overall secondary structure of the mutant variants was unaltered but local conformational changes were observed. Interestingly, both the mutant variants exhibited dominant negative behavior when expressed in cells with a wild type copy of Dnm1. To our knowledge we report for the first time a single residue mutation that does not alter the localization of Dnm1 but makes it non-functional in a dominant negative manner. Our data highlights that mutations in the same domain can exhibit variable effects.

# UNDERSTANDING PROTEIN EXPRESSION LIMITS AND TOXICITY IN BUDDING YEAST

Shotaro Namba, Hisao Moriya

Okayama University, Environmental and Life Science, Okayama, Japan

A valuable means of understanding the characteristics of an organism is to understand abilities and constraints. In particular gene expression levels are an important determinant of the phenotype of an organism. Therefore, identifying the constraints of gene expression, i.e., the lower and upper limits, is crucial in defining cell growth and traits.
The budding yeast *Saccharomyces cerevisiae* is an ideal model organism for studies on its gene expression due to its ease of genetic control and abundant genetic information. From previous studies, the lower limit of gene expression, i.e., the minimum expression level required for the growth of each gene, is relatively simple to measure. This is because the criteria for the lower limit are clear. The lower limit is the complete absence of gene expression, i.e., zero expression level. Therefore, the lower limit can be measured by creating a deletion strain or by replacing the inducible promoter.
However, measuring the "upper limit" of gene expression is challenging. This is because the upper limit of gene expression can only be measured by increasing expression levels until growth defects occur. Previous studies have observed growth inhibition due to overexpression in only about 1,000 of the 6,000 genes in yeast. The reason why growth inhibition has not been observed for many genes is thought to be that the expression systems used in previous studies do not have enough expression levels.
In this study, we constructed a powerful expression induction system by using WTC846, in combination with a plasmid copy number increase by the genetic tug-of-war method. Using this expression method, we constructed a system that allows us to investigate the expression limits of any protein by expressing a gene fused to a fluorescent protein and observing the dynamics of the target protein during growth defects.
First, to confirm that the expression system we constructed had enough expression level, we expressed moxGFP, which is considered one of the most non-harmful proteins in yeast. The results showed that expression of moxGFP caused growth defects. This indicates that the constructed system has enough expression levels. Next, the genes encoded on the budding yeast I chromosome were comprehensively examined. As a result, growth inhibition was observed for almost all analyzed proteins, and their maximum expression levels were obtained. In addition, several proteins were found to induce abnormal structures in cells when overexpressed.
This method allowed us to evaluate the maximum expression levels and intracellular dynamics of all proteins in budding yeast. In the future, we will analyze the mechanism of toxicity of chromosome I genes in terms of localization, function, codon bias, and structure. In addition, we will focus on genes with relatively low toxicity and elucidate the factors responsible for their non-toxicity.

# IDENTIFICATION OF POTENTIAL THERAPEUTIC LEAD COMPOUNDS TARGETING SERINE-PALMITOYLTRANSFERASE

Lien Thi Kim Pham[1], Mami Yoshimura[1], Yoko Yashiroda[1], Sheena Li[1], Matej Usaj[1,2], J. Rafael Montenegro Burke[2], Minoru Yoshida[1], Charles Boone[1,2]

[1]RIKEN, Center for Sustainable Resource Science, Wako, Japan, [2]University of Toronto, Department of Molecular Genetics, Toronto, Canada

As a critical enzyme of sphingolipid metabolism, serine-palmitoyltransferase (SPT), is conserved from yeast to humans. Recently, a genetic form of a rare childhood form of amyotrophic lateral sclerosis (ALS) has been found to be linked to variations in the human SPTLC1 gene, which encodes a subunit of SPT. In particular, these ALS-associated mutations enhanced SPT activity. Thus, a direct inhibitor of SPT may hold promise for future therapeutic studies. However, thus far, no selective SPT inhibitors have been prioritized for clinical development. To link bioactive compounds to their cellular targets, our group employs chemical genomics (CG) screening platforms, which measure the fitness of mutant yeast strains in the presence of bioactive compounds. Our CG platforms score the fitness of hundreds of different pooled yeast strains in parallel through a barcode sequencing approach, which ultimately generates a CG profile that is predictive of the compound mode of action. For comprehensive CG screening, we employ several different sets of mutant yeast strains, including both ~1000 heterogeneous (HET) diploid mutants and ~ 1000 temperature-sensitive (TS) haploid mutants, each covering the yeast essential gene set, as well as ~5000 viable haploid (HAP) deletion mutants, covering the yeast nonessential gene set. We use our powerful platform to assay various compound libraries, including those composed of largely uncharacterized bioactive compounds derived from the RIKEN Natural Product Depository and the University of Tokyo Drug Discovery Initiative libraries. In this study, we focus on identifying new potent compounds that target SPT selectively. We identified a compound, NPL00587, that is not structurally related to known SPT inhibitors, such as myriocin or sphingofungin B, which resemble sphingosine derivatives. Like myriocin, the most extreme negative HET CG interactions were with coding genes encoding yeast SPT, LCB1 and LCB2. Ultimately, we validated and characterized NPL00587 as a novel SPT inhibitor through detailed yeast and human cell CG analyses, drug-resistant mutant analysis, as well as targeted biochemical experimentations, such as LC-MS-based-sphingolipid profiling.

# YEAST AS A MODEL TO STUDY α-SYNUCLEIN MEDIATED CELLULAR CHANGES

Neha Joshi*, Tanveera R Sarhadi*, Atchaya Raveendran

Indian Institute of Technology, Guwahati, Biosciences and Bioengineering, Guwahati, India

α-synuclein aggregation is the hallmark feature of Parkinson's disease. Both familial and sporadic forms of the disease exhibit this feature. Several mutations have been identified in patients and are associated with the disease pathology. We have used site-directed mutagenesis to generate α-synuclein mutant variants tagged with GFP. Fluorescence microscopy, flow cytometry, western blotting, cell viability and oxidative stress analysis were performed to investigate the effect of two less studied α-synuclein variants. In this study we characterized two less studied α-synuclein mutations, A18T and A29S, in the well-established yeast model. Our data shows variable expression, distribution and toxicity of the protein in the mutant variants A18T, A29S, A535T and WT. The cells expressing the double mutant variant A18T/A53T showed the most increase in the aggregation phenotype and also depicted reduced viability suggesting a more substantial effect of this variant. The outcome of our study highlights the variable localization, aggregation phenotype and toxicity of the studied α-synuclein variants. This underscores the importance of in-depth analysis of every disease-associated mutation which may result in variable cellular phenotype. In addition, our data also suggests an effect of α-synuclein expression on mitochondrial morphology and peroxisome number.

*authors contributed equally

# THE MITOCHONDRIAL HSP70 CONTROLS THE ASSEMBLY OF THE F1FO-ATP SYNTHASE

Jiyao Song, Thomas Becker

Institute for Biochemistry and Molecular Biology, Faculty of Medicine, Bonn, Germany

The F1FO-ATP synthase utilizes the proton gradient that is generated by the respiratory chain to produce the bulk of cellular ATP. The yeast F1FO synthase contains 17 subunits. Three of these are encoded in the mitochondrial DNA, while the remaining fourteen subunits are synthesized in the cytosol. The nuclear encoded subunits are synthesized on cytosolic ribosomes and then imported into mitochondria via the translocase of the outer membrane (TOM complex) and the presequence translocase (TIM23 complex). The import of matrix-localized subunits of the ATP synthase additionally depends on the activity of mitochondrial Hsp70 (mtHsp70), which is the core subunit of the presequence translocase-associated motor (PAM). Here, we report a dual role of mtHsp70 in the formation of the ATP synthase. mtHsp70 cooperates with the known assembly factors of the F1FO -ATP synthase, Atp11 and Atp12, to build up the F1 part. Additionally, mtHsp70 functions as a pool for Atp5 and works together with INAC (Ina17 and Ina22) to promote the association of the peripheral stalk with the catalytical head. Inactivation of mtHsp70 leads to integration of an assembly-defective Atp5 variant into the mature complex, reflecting the quality control function of this chaperone. Thus, mtHsp70 acts as an assembly and quality control factor in the biogenesis of the F1FO -ATP synthase.

# A GENETIC APPROACH TO IDENTIFY A NOVEL TORC2 DOWNSTREAM SIGNALING PATHWAY

Ken-taro Sakata, Keisuke Hashii, Koushiro Yoshizawa, Takayoshi Sakamoto, Naotaka Tanaka, Mitsuaki Tabuchi

Kagawa University, Applied Life Science, Takamatsu, Japan

The budding yeast *Saccharomyces cerevisiae* has three distinct plasma membrane domains, MCC (Membrane Compartment occupied by Can1), MCT (Membrane Compartment occupied by TORC2), and MCP (Membrane Compartment occupied by Pal). MCC corresponds to inner membrane furrows termed eisosome. Eisosome contains various membrane associated-proteins, transporters, and tetraspan membrane proteins, which are considered to contribute to normal eisosome structure and function. Pill is a core eisosome protein and is responsible for MCC-invaginated structures. In addition, six tetraspan membrane proteins (6-Tsp) are localized in the MCCs and classified into two families, the Sur7 family, and the Nce102 family. To understand the coordinated function of these MCC proteins, single and multiple deletion mutants of Pill and 6-Tsp were generated. These deletion mutant cells analyzed the structure of the eisosome and assayed growth under various stress conditions. Genetic interaction analysis indicated that the Sur7 family and Nce102 function in stress tolerance and normal eisosome assembly, respectively, by cooperating with Pill. To further understand the role of MCCs/eisosomes in stress tolerance, we screened for suppressor mutants using the SDS-sensitive phenotype of *pill*Δ *6-tsp*Δ cells. The growth defect on the SDS-contained medium was suppressed by the reduction of function of Tor2, which is the component of the Tor kinase complex 2 (TORC2). This data indicated that the SDS sensitivity in *pill*Δ *6-tsp*Δ cells is caused by hyperactivation of TORC2-Ypk1 signaling. Interestingly, inhibition of sphingolipid metabolism, a well-known downstream pathway of TORC2-Ypk1 signaling, did not rescue the SDS-sensitivity of *pill*Δ *6-tsp*Δ cells, indicating that SDS-sensitivity of *pill*Δ *6-tsp*Δ cells caused by other TORC2-downstream pathways. To explore the unknown TORC2 downstream pathways, we further isolated *sde* mutants (Suppressor for SDS sensitivity of Eisosome deletion mutants) and classified them into 8 complementation groups. This genetic screening proved to be an efficient way to explore downstream pathways of the TORC2-Ypk1 pathway, as mutations in all essential genes of TORC2 components (*TOR2*, *AVO1*, *AVO3*, *LST8*), and *YPK1* were isolated. This screening also identified a complementation group distinct from genes on the TORC2-Ypk1 signaling pathway, related to the phosphoinositide-signaling pathway. Biochemical analysis suggested that the phosphoinositide signaling pathway functions downstream of the TORC2-Ypk1 signaling pathway. Our results may uncover the novel link between TORC2- and phosphoinositide-signaling pathways.

# ANALYSIS OF THE FISSION YEAST POF1 ON THE ACCUMULATION OF RECOMBINATION INTERMEDIATES

Jiashen Tang, Mikio Nakamura, Masaru Ueno

Hiroshima University, Graduate School of Integrated Sciences for Life, Higashihiroshima, Japan

Accurate chromosome segregation during the M phase is an essential process for the stable maintenance of chromosomes and genetic material, and it is strictly controlled. Recombination intermediates are usually temporary DNA structures generated and resolved during DNA homologous recombination repair processes. The connection between sister chromatids, such as recombination intermediates, can cause chromosome segregation defects, leading to cell death or cancer (Nicholas M et al., EMBO,2012). Researching recombination intermediates may deepen our understanding of carcinogenesis and drug resistance in cancer. Therefore, in our laboratory, we study the factors that are involved in the regulation of the accumulation of recombination intermediates.

Fission yeast Rqh1 is a helicase involved in suppressing inappropriate DNA recombination initiation and the resolution of recombination intermediates. Deletion of rqh1 leads to frequent DNA recombination and accumulation of recombination intermediates. Fission yeast Pof1 is an essential fission yeast F-box protein that recognizes proteins for degradation.

Due to HU (DNA replication inhibitor) treatment, rqh1 mutant strains accumulate recombination intermediates on the chromosomes. In the rqh1 mutant strain, the frequency of this chromosome segregation defect increases upon HU treatment. And overexpression of enzyme that resolve recombination intermediates suppress chromosome segregation defects. (Claudette L. Doe et al., EMBO J, 2000). It is inferred that recombination intermediates may be responsible for inducing chromosome segregation defects. We found that treating rqh1-hd (helicase dead) strain with HU becomes sensitive to TBZ (microtubule inhibitor). This experiment aims to elucidate the mechanism of suppressing HU・TBZ sensitivity of rqh1-hd by pof1 mutation.

We also found that the HU・TBZ sensitivity in the rqh1-hd strain is attenuated by the pof1-A81T mutation. However, the mechanism by which pof1-A81T mutation suppresses HU・TBZ sensitivity in rqh1-hd is still not clear. The aim of this experiment is to elucidate the mechanism of suppressing HU・TBZ sensitivity of rqh1-hd by pof1 mutation.

We found that the pof1 mutation suppresses the segregation defect of Gar2 (colocalizes in nucleolus which could reflect rDNA segregation defect) in rqh1-hd strain under HU treatment. Our results suggest that the pof1-A81T mutation suppresses the accumulation of recombination intermediates at rDNA in the rqh1-hd strain. In contrast, the pof1 mutation does not suppress the TBZ sensitivity of pot1 rqh1-hd strain, where recombination intermediates accumulate at telomeres. The results of this study show that the pof1 mutation suppresses the phenotype at rDNA in rqh1-hd mutants but does not suppress the phenotype at telomeres.

# CATALYTICALLY INACTIVE CAS9 ATTENUATES DNA END RESECTION IN *SACCHAROMYCES CEREVISIAE*

Suchin Towa, Satoshi Okada, Takashi Ito

Kyushu University Graduate School of Medical Sciences, Biochemistry, Fukuoka, Japan

Catalytically inactive Cas9 (dCas9) has various unique applications. However, it remains elusive how dCas9 affects endogenous events on the genomic DNA. We previously reported that dCas9 impairs DNA replication fork progression in *Saccharomyces cerevisiae*. Here we examined whether dCas9 affects the end resection induced by DNA double-strand breaks (DSBs). We first used the single-stranded DNA (ssDNA)-specific quantitative PCR (qPCR) assay to demonstrate that dCas9 limits the extent of end resection from DSB sites. We next developed a convenient ssDNA-specific microscopic assay using the ssDNA-binding protein Rfa1 fused with the fluorescent protein mNeonGreen. While the microscopic assay was less sensitive than the qPCR assay, both consistently supported dCas9-induced attenuation of end resection. We finally used a genetic assay based on an elevated susceptibility of ssDNA to bisulfite-induced mutagenesis to examine the impact of dCas9 binding. The genetic assay showed a good correlation with the qPCR assays. Hence our findings strongly suggest that dCas9 can impede the progression of DNA end-resection machinery. We expect a potential utility of dCas9 as a modulator of end resection, a critical determinant of DSB repair pathway choice.

# INTERTUMORAL AND INTRATUMORAL HETEROGENEITY OF HYPOXIA RESPONSE IN HEPATOCELLULAR CARCINOMA

Gengchao Wang, Chun-Ming Wong

State Key Laboratory of Liver Research, Department of Pathology, The University of Hong Kong, Hong Kong, Hong Kong

Hypoxia is associated with tumor development, and it affects many biological processes, such as angiogenesis, metabolism, and immune escape. Hypoxia stabilizes hypoxia-inducible factors (HIFs), which act as transcription factors to induce gene expression by binding to the hypoxia-responsive element (HRE) sequence. Interestingly, we found that hypoxia induces different subsets of genes in different liver cancer cell lines. However, the causes and consequences of the heterogeneity response to hypoxia remain unclear. Here, using integrated-omics studied we showed that the diverse hypoxia responses may be pre-determined by the epigenetic landscape, especially DNA methylation, that modulates the binding of HIFs to the promoter HRE. Using bulk RNA-Seq data in TCGA and our in-house single-cell RNA-Seq data, we revealed the intertumoral and intratumoral heterogeneity of hypoxia response in human liver cancer. In addition, we found that the heterogeneity of hypoxia response might influence the immune microenvironment. A subset of immune regulators was differentially induced by hypoxia in different tumors and their expressions were negatively correlated with tumor CD8+ T-cell infiltration, indicating the possible impact of heterogeneity of hypoxia response on the tumor immune microenvironment. Our results revealed the intertumoral and intratumoral heterogeneity of hypoxia response in HCC and its effect on the tumor immune microenvironment. This study contributes to a better understanding of the role of heterogeneity in response to hypoxia during tumor development in HCC and its potential as a biomarker to guide immunotherapy.

# ELUCIDATION OF THE ROLE OF BQT4 ON DYNAMICS IN NUCLEUS IN S.POMBE.

Kaiyu Wang, Masaru Ueno

Hiroshima university, Graduate School of Integrated Sciences for Life, Higashi-Hiroshima City, Hiroshima, Japan

In S. pombe, inner-nuclear-membrane protein Bqt4 plays important role in attaching telomere to nuclear envelope. We found the decrease in MSD (Mean square displacement) between nucleolus and (SPB) spindle pole body in bqt4Δ. Based on this and other data, we hypothesize that the nucleolus movement of nucleolus in bqt4Δ changes. We are trying to confirm this hypothesis and study the mechanism of the changed nucleolus movement, which might also involve in human cancer cell development.
Positive correlation between SPB and nucleolus movement spectrogram obtained from performing Fourier transform on the motion trajectory plot of nucleolus and SPB suggests the synchronized movement of nucleolus and SPB, which might be caused by the NE properites: acting as mechanic force transductor or certain polymerization pattern between opposite site microtubules.
In bqt4Δ, nucleolus exhibit more pronounced motion than WT in the direction perpendicular to the long axis of cells, which is solidate evidence for the of nucleolus movement change in one of parameters in bqt4Δ. Nucleolus movement change in bqt4Δ maybe microtubule dependent, supported by the fact that differences in MSD of bqt4Δ and WT is diminished when SPB movement is stopped by MBC.Strikingly, nucleolus movement is also microtubule-dependent, since the norm of nucleolus-movement-vector in the direction parallel to long axis of cell decreased.
bqt4Δ group showed milder MSD increase than WT when SPB movement is enhanced by NH4Cl, suggests that NH4Cl and Bqt4 shows functional interaction. Our interpretation of this resiult is either that nucleolus can easily follow the movement of SPB to maintain the certain distance between SPB and nucleolus in bqt4Δ or that the relative position of SPB and nucleolus is more fixed in bqt4Δ.
Strains lacking both Rap1 and DNA binding ability which are localized on N-terminal domain ( NTD) of bqt4 or bqt4 NTD-truncation-mutant did not show bqt4Δ-like-MSD,suggesting that loss of both DNA binding and Rap1 binding in Bqt4 is insufficient to decrease MSD to similar level with bqt4Δ.
In conclusion, C-terminal domain (CTD) of Bqt4 might possess an unknown non-telomeric function, which is responsible for MSD decrease in bqt4Δ.

# A NANOBODY COLLECTION FOR STUDYING THE SORTING MECHANISMS OF VPS74P

Ziyun Ye, David K Banfield

The Hong Kong University of Science and Technology, Division of Life Science, Kowloon, Hong Kong

Vps74p is a peripheral membrane protein that functions as a sorting receptor for the COPI-mediated retrograde transport of various Golgi enzymes such as glycosyltransferases. The structure and functions of Vps74p are evolutionarily conserved. Interestingly, one human homolog of Vps74p, GOLPH3 is an oncogene which is amplified in a variety of tumors. GOLPH3 amplification is a poor prognostic marker for cancer treatment. Thus, understanding the sorting mechanism of Vps74p family proteins not only provides insights into the organization and maintenance of the Golgi, but may also shed light on protein targets for novel cancer therapeutics. Here, we describe the isolation of a suite of nanobodies (15 kDa single-domain antibodies) against Vps74p to facilitate the elucidation of this receptor's sorting mechanism. Some of the anti-Vps74p nanobodies also cross-react with human GOLPH3 and GOLPH3L. We subjected the suite of nanobodies to an in vivo functional assay to identify those that could block the critical functions of Vps74p. Yeast cells expressing these nanobodies (termed functional blockers) showed the mislocalization of Vps74p-dependent Golgi clients and the loss of membrane association of Vps74p, suggesting that the functional blockers bind to the membrane-facing surface of Vps74p. The membrane-facing surface most likely contains the client-binding sites, and indeed some functional blockers reduced client-binding to Vps74p in biochemical assays. In addition, the functional blocker that also cross-reacts with GOLPH3 and GOLPH3L could ablate the interaction between Vps74p and Arf1p, an interaction critical for client sorting via the COPI coat. The anti-Vps74p nanobody collection represents a toolkit with applications in the identification of binding sites, protein localization imaging, structure determination, and potential GOLPH3-targeted cancer therapies.

# NAVIGATING TRAFFIC AT REPLICATION FORKS: REPLISOME-HISTONE CHAPERONE COLLABORATION IN GUIDING THE HISTONE PARTITIONING

Qing Li

Peking University, School of Life Sciences, Beijing, China

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.

# CHROMATIN STRUCTURE IN FISSION YEAST SPORES

Aki Hayashi[1], Reiko Nakagawa[2], Yasuyuki Ohkawa[3], Jun-ichi Nakayama[1]

[1]Division of Chromatin Regulation, National Institute for Basic Biology, Okazaki, Japan, [2]Laboratory for Cell-Free Protein Synthesis, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan, [3]Division of Transcriptomics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

Gametogenesis is an essential process for sexually reproducing organisms to produce haploid gametes. During gametogenesis, diploid cells reorganize parental genomic information through meiotic processes, allowing the offspring to acquire genetic diversity and the ability to adapt to variable environments in the next generation. Along with the genomic DNA, epigenetic information is thought to be inherited by the next generation. However, how epigenetic marks are regulated during gametogenesis remains to be fully elucidated. To gain the insight into epigenetic inheritance in gametes, we focused on fission yeast spores as a model for gametogenesis. We isolated fission yeast spores by density gradient centrifugation and performed proteomic and transcriptome analyses of the spores. Both analyses indicate that fission yeast spores are in a dormant state. We then analyzed histone modifications by ChIP-seq analysis. Histone H3 lysine 9 methylation (H3K9me), a mark of transcriptional repression, is localized to heterochromatin regions and is not significantly different from that of vegetative cells. Interestingly, we found that histone H3 lysine 4 methylation (H3K4me), a mark associated with transcriptional activity, shows a distinct localization pattern in spores. In this talk, I will discuss about the importance of histone modifications in spores for the inheritance of epigenetic information.

# SWI2 ACTIVATES RAD51 STRAND EXCHANGE ACTIVITY AND IS INVOLVED IN DONOR CHOICE FOR GENE CONVERSION OF MATING TYPE SWITCHING IN FISSION YEAST

Hiroshi Iwasaki

Tokyo Institute of Technology, Cell Biology Center, Yokohama, Japan

In fission yeast *Schizosaccharomyces pombe*, a homothallic haploid cell expresses either P or M mating type, determined by the active *mat1* cassette. The mating type is switched through Rad51-driven gene conversion of *mat1*, using a donor cassette, *mat2-P* or *mat3-M*. Central to this process is the Swi2-Swi5 complex, which designates a preferred donor per cell type by using one of two Swi2-dependent recombination enhancers: *SRE2* or *SRE3*. Consequently, M cells choose *mat2-P* and P cells choose *mat3-M* for gene conversion. Interestingly, the C-terminal half of the Swi2 sequence shows sequence similarity with Sfr1. Sfr1 also forms a complex with Swi5 that acts as a Rad51 mediator for general homologous recombination and repair, but not mating-type switching. This study identifies two key motifs in Swi2: a Swi6 (HP1)-binding site and two AT-hook DNA binding motifs. Genetic analysis shows these hooks are needed for Swi2 localization at *SRE3* or *SRE2* to select the donor in P or M cells. Furthermore, the Swi2-Swi5 complex promotes Rad51-driven strand exchange in vitro. In conclusion, our results suggest the Swi2-Swi5 complex localizes to recombination enhancers via a cell-specific mechanism, stimulating Rad51-driven gene conversion at the localization site for mating-type switching.

# TRANSCRIPTIONAL RESTART CAUSES CHROMOSOMAL REARRANGEMENTS AT CENTROMERES

Ran Xu[1,2], Cristal Tang[1], Takuro Nakagawa[1,2]

[1]Osaka University, Department of Biological Sciences, Graduate School of Science, Toyonaka, Japan, [2]Osaka University, Forefront Research Center, Graduate School of Science, Toyonaka, Japan

Centromeres play a crucial role in chromosome segregation. However, centromeres contain repetitive DNA sequences in many eukaryotes and are prone to suffer gross chromosomal rearrangements (GCRs). Heterochromatin suppresses centromeric GCRs by repressing transcription of centromere repeats. However, how transcription causes GCRs remains unclear. Here, we found that transcriptional restart causes GCRs via DNA-RNA hybrid formation in the fission yeast *Schizosaccharomyces pombe*. DNA-RNA immunoprecipitation (DRIP) assay showed that loss of Clr4 H3K9 methyltransferase essential for heterochromatin assembly increased DNA-RNA hybrids at centromeres. Overproduction of RNaseH1 reduced DNA-RNA hybrids and GCRs, demonstrating that DNA-RNA hybrids mediate GCRs. Tfs1/TFIIS transcription factor and Ubp3 ubiquitin hydrolase promote transcriptional restart following RNA polymerase II pausing/backtracking. *tfs1* and *ubp3* mutations specifically reduced DNA-RNA hybrids and GCR rates, suggesting that transcriptional restart causes centromeric GCRs through DNA-RNA hybrid formation. Rad51 was dispensable for GCRs in *clr4*Δ cells. However, the *rad52-R45K* mutation that impairs single-strand annealing (SSA) activity reduced DNA-RNA hybrids and GCRs in *clr4*Δ cells. These data suggest that transcriptional restart induces DNA/RNA-loop (DR-loop) formation, leading to centromeric GCRs. Our findings have uncovered a role of transcriptional restart in genome instability.

# LAGGING STRAND RATHER THAN LEADING STRAND TELOMERES CAUSE THE END REPLICATION PROBLEM

Tiantian Ye[1], Li-Juan Fu[2,1], Jia-Cheng Liu[1], Zhi-Jing Wu[3], Jin-Qiu Zhou[1,2,3]

[1]CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, University of Chinese Academy of Sciences, Shanghai 200031, China, [2]School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China, [3]School of Life Science, Hangzhou Institute for Advanced Study, University of the Chinese Academy of Sciences, Hangzhou 310024, China

Telomeres, the physical ends of linear eukaryotic chromosomes, are essential for chromosome integrity and genome stability. Without telomerase-mediated telomere elongation, telomeric DNA appears to gradually shorten during successive cell divisions presumably because the RNA primer of the very terminal Okazaki fragment in the lagging strand is removed, resulting in incomplete replication of the parental DNA, referred to as “end replication problem”. Additionally, the telomere produced by the replication of the leading strand is generally believed to undergo end-resection, fill-in synthesis and RNA primer removal processes, accentuating the end replication problem. Using a *de novo* telomere induction system in *Saccharomyces cerevisiae*, we were able to detect the dynamics of telomere 3’-overhang at near nucleotide resolution. We found that the *de novo* telomere induced by Cas9 is refractory to nuclease resection, and the very end is efficiently capped by Yku complex. Analysis of 3’-overhang emergence during a single-round of DNA replication showed that the leading strand telomere possesses a stable blunt end, which is dependent on Yku. In addition, the lagging strand telomere contains an ~10-nt 3’-overhang derived from the RNA primer removal, which mainly requires Rnh201 endo-ribonuclease. Our results clarify that the completely replicated leading strand telomere possess a stable blunt end sheltered by Yku, and the primary cause of the end replication problem is the incomplete replication of the lagging strand.

MECHANICS OF CELLULAR ORGANIZATION

Fred Chang

UCSF, Cell and Tissue Biology, San Francisco, CA

Although the cytoplasm is often depicted as a watery solution, it probably more resembles lumpy oatmeal: a dense, viscoelastic, heterogenous matrix. We study how physical properties of the cytoplasm affect intracellular dynamics and organization, using fission yeast *S. pombe* as a model system. I will present our recent results on the mechanism governing the size of the nucleus. The size of the nucleus scales robustly with cell size so that the nuclear-to-cell volume ratio (N/C ratio) is maintained during cell growth. The mechanism responsible for this scaling remains mysterious. Indeed, previous studies show that the N/C ratio is not determined directly by DNA amount. Our findings reveal that the size of the nucleus is determined by a balance of colloid osmotic pressures generated by the collections of macromolecules in the nucleoplasm and cytoplasm

# EXPLOITING DIVERGENT BIOLOGY OF TWO FISSION YEASTS TO UNDERSTAND MEMBRANE FUNCTION

Snezhana Oliferenko[1,2]

[1]The Francis Crick Institute, London, United Kingdom, [2]King's College London, Randall Centre for Cell and Molecular Biophysics, London, United Kingdom

Biological membranes are semi-permeable lipid barriers delimiting cells and subcellular compartments. By recruiting and scaffolding specific proteins and protein complexes, membranes also serve as platforms for cellular communication, signalling and metabolism. The specific features of the membrane depend on its lipid composition. I will present our recent work aimed at understanding how lipid metabolism impacts on membrane function and cellular physiology using comparative and synthetic approaches in two related fission yeast species with different lifestyles. Briefly, we show that a popular model system *Schizosaccharomyces pombe* and its less known relative *Schizosaccharomyces japonicus* exhibit strikingly different membrane lipid composition and provide the mechanistic explanation for this divergence. I will further argue that these differences in lipid metabolism may be at root of the profound changes to cellular physiology that occurred in the evolution of the fission yeast clade.

# ARTIFICIAL KAROTYPE ENGINEERING REVEALS HIGH PLASTICITY OF EUKARYOTIC GENOME

Xin Gu, Yuang Wang, Xueting Zhu, Jin-Qiu Zhou

Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai 200031, China

The genetic information of eukaryotes is buried in chromosomes. Different organisms usually contain different numbers of chromosomes. Presumably the organization and/or number of chromosomes in a particular species have evolved to well adapt to the living environment. Artificial chromosome fusions in both *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* yeasts creat phenotypically healthy single-chromosome cells. The mouse haploid embryonic stem cells (haESCs) containing chromosome 15-17 fusion is pluripotent to generating heterozygous (2n=39) and homozygous (2n=38) mice. Mice containing the fusion chromosome are fertile, and their representative tissues and organs display no phenotypic abnormalities, suggesting unscathed development. These studies reveal the configuration robustness of eukaryotic genomes, and reorganization of genome (chromosomes) can be readily tolerated even in mouse development.

# CONSECUTIVE AMINO ACID SEQUENCES CAN BE TOXIC

Naoki Kitamura[2], Hisao Moriya[1]

[1]Okayama University, Agriculture, Okayama, Japan, [2]Okayama University, Health system, Okayama, Japan

Domains consisting of a single sequence of amino acids (referred to here as "PolyX") are known for their biological functions and association with diseases, and are also used in cell engineering.

In the amino acid sequence in the proteome of budding yeast (*S. cerevisiae*), only nine PolyXs with 10 or more consecutive amino acids are found, and their number is highly biased toward D, E, N, Q, and S.

Thus, there is a large bias in the PolyXs used by organisms, and certain PolyXs seem to be rather excluded from the proteome.

One possible reason for the absence of PolyXs on the proteome could be that they have a negative effect on cells, i.e., they are toxic.

To test this possibility, we added 10 consecutive amino acids to the C-terminus of GFP, expressed it in yeast cells to the limit of growth inhibition by the genetic tug-of-war method (Moriya *et al.*, *PLOS Genet*. 2006), and evaluated its toxicity.

We found a high correlation between the toxicity of this experimentally obtained GFP-Poly10X and the frequency of PolyX in the proteome, suggesting that the toxicity of a particular PolyX in the proteome is the reason for its absence.

# MAPPING GENETIC INTERACTION NETWORKS IN YEAST AND HUMAN CELLS

Charles M Boone

University of Toronto, Donnelly Centre for Cellular and Biomolecular Research, Toronto, Canada

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 and coherent sets of genetic interactions connect protein complex and pathway modules to map a functional wiring diagram of the cell. We've examined trigenic interaction networks and how different environments modulate the global yeast genetic interaction network. We are now exploring how different genetic backgrounds influence the global yeast genetic network. To test for conservation of the general principles of genetic networks, we are utilizing CRISPR-Cas9 technology to conduct genome-wide screens and map genetic interactions in haploid human cells.

# EXTRACELLULAR EXPORT OF RNA-DERIVED MODIFIED NUCLEOSIDES IN BUDDING YEAST

Mitsutaka Kubota[1], Hiroko Miyamoto[2], Fan-Yan Wei[2], Koji Okamoto[1]

[1]Osaka University, Graduate School of Frontier Biosciences, Osaka, Japan, [2]Tohoku University, Institute of Development, Aging and Cancer, Sendai, Japan

In eukaryotic cells, intracellular degradation serves as a quality and quantity control mechanism of cellular components, thereby contributing to cell homeostasis. Although most catabolites are recycled into de novo synthesis, those containing irreversible modifications are non-recyclable for anabolic reactions and thus need to be exported into the extracellular space. Recent studies reveal that nucleosides are generated through autophagy-dependent RNA degradation and released from the cytoplasm. However, molecular mechanisms underlying extracellular unmodified/modified nucleoside export and its physiological implications remain largely unknown. To explore these fundamental issues, we performed a genome-wide screen for non-essential gene deletion mutants of the budding yeast *Saccharomyces cerevisiae* defective in extracellular RNA-derived nucleoside export. Using comprehensive mass spectrometry for 30 different unmodified/modified nucleosides released into the culture supernatant of cells under nitrogen starvation, we identified a myriad of Nex (nucleoside export) protein candidates acting in autophagy, vacuole-related processes, RNA turnover/processing, ribosome biogenesis, metabolite transport, membrane trafficking, mitochondrial functions, and others. Notably, those Nex proteins are classified into three groups related to either tRNA or rRNA, and common for all RNA species. We also established a mass spectrometry-based monitoring system to investigate extracellular export of a mitochondrial tRNA-specific modified nucleoside and found that it was released into the culture supernatant in a manner dependent on Atg32, a protein required for selective autophagy of mitochondria. Experiments are currently underway to elucidate how distinct RNA species are efficiently degraded via autophagy-related processes and how the resulting free nucleosides are properly exported into the extracellular space.

# MODE-OF-ACTION ANALYSIS OF INHIBITORS TARGETING ERGOSTEROL BIOSYNTHESIS USING YEAST CHEMICAL GENOMICS

Yoko Yashiroda[1], Mami Yoshimura[1], Masaya Usui[2], Hiromi Kimura[1], Luis Alberto Vega Isuhuaylas[3], Lien Thi Kim Pham[1], Takashi Niwa[4,5,6], Ikko Takahashi[1], Takamitsu Hosoya[5,6], Laurean Ilies[1], Minoru Yoshida[1,7], Charles Boone[1,3]

[1]RIKEN, Center for Sustainable Resource Science, Wako, Japan, [2]RIKEN, Center for Brain Science, Wako, Japan, [3]University of Toronto, Donnelly Centre, Toronto, Canada, [4]Kyushu University, Graduate School of Pharmaceutical Sciences, Fukuoka, Japan, [5]RIKEN, Center for Biosystems Dynamics Research, Kobe, Japan, [6]Tokyo Medical and Dental University, Institute of Biomaterials and Bioengineering, Tokyo, Japan, [7]The University of Tokyo, Graduate School of Agricultural and Life Sciences, Tokyo, Japan

Ergosterol is a critical sterol in the cell membranes and its synthesis is tightly regulated by known enzymes in yeast. The removal of two methyl groups at the C-4 position of a sterol intermediate is an essential step in ergosterol biosynthesis. In the budding yeast *Saccharomyces cerevisiae*, it has been demonstrated that the three endoplasmic reticulum membrane-localized enzymes Erg25 (C-4 sterol methyl oxidase), Erg26 (C-4 decarboxylase/C-3 sterol dehydrogenase), and Erg27 (C-3 keto reductase) are involved in the demethylation reaction that removes the two methyl groups at the C-4 position of 4,4-dimethylzymosterol (4,4-DMZ): (i) Erg25 converts the C-4 methyl group to a carboxylic acid; (ii) Erg26 generates a keto group at the C-3 position and removes the carboxylic acid from C-4, and finally; (iii) Erg27 restores the hydroxyl group at the C-3 position. This sequence of reactions is repeated twice to convert 4,4-DMZ into zymosterol. All three enzymes are essential for cell growth. During screening of bioactive compounds from the RIKEN in-house chemical library, we found that two boron-containing compounds, CP90399 and CP91389, that exhibited growth inhibitory activity against *S. cerevisiae*. Chemical genomics (CG) methodology, which relies on quantifying how precise genetic perturbations affect the response of cells to a bioactive compound, provides a powerful systematic approach to identify the mode-of-action of bioactive compounds. We established a comprehensive yeast cell-based CG analysis pipeline that employs various diagnostic sets of the *S. cerevisiae* mutants (Persaud *et al.*, 2022, *Cell Chem Biol.*; Iyer *et al.*, 2023, *Cell Chem. Biol.*). In particular, we performed comprehensive CG screens for the CP compounds using strain sets that span ~1,000 essential yeast genes, including a set of heterozygous diploid deletion mutant (HET) strains and a set of gene overexpression (MoBY) strains. We found that the *ERG25* heterozygous disruption strain was the most sensitive and that an *ERG25* gene overexpression strain was the most resistant. In addition, the initial substrate 4,4-DMZ accumulated in cells upon treatment with the CP compounds compared to the DMSO control treatment. These phenotypes are similar with the ones for a known Erg25 inhibitor PF1163A, suggesting that the target of the CP compound is Erg25. We will present our latest results including isolation of drug resistant mutants to further address the precise mode-of-action of the CP compounds.

# A CHEMICAL GENETIC SCREEN FOR DRUGS THAT AFFECT MICROTUBULE ORGANIZATION OF FISSION YEAST

Masamitsu Sato[1,2], Mamika Minagawa[1], Mika Toya[1,3], Shigehiro Kawashima[4]

[1]Waseda University, Laboratory of Cytoskeletal Logistics, Tokyo, Japan,
[2]Waseda University, Inst. for Medical-Oriented Struct. Biol., Tokyo, Japan,
[3]Waseda University, Global Center for Sci. and Eng., Tokyo, Japan,
[4]University of Tokyo, Grad. Sch. of Pharm. Sci., Tokyo, Japan

Drugs that affect cellular microtubule organization have been used as fungicides or anti-cancer drugs, as they inhibit mitotic spindle assembly and induce fatal errors in chromosome segregation. Here, we present a screen for compounds that target microtubules, in the combination of chemistry and genetics using the fission yeast *Schizosaccharomyces pombe*. Our aim to isolate new drugs from a library that reduce viability of *S. pombe* specifically owing to microtubule damages.

We first employed the strain harboring the MDR-sup background as a host strain of the screening, as the strain displays increased sensitivities to multiple drugs (Kawashima et al. 2012; Aoi et al. 2014). The genetic strategy was taken to efficiently screen the mega-library comprised of ~100,000 compounds for those that reduced viability of the MDR-sup strain. Compounds that affected viability were subsequently tested for the secondary screen. The second strategy taken at this step is to exclude 'old' drugs that have been already shown to affect microtubules. We used the β-tubulin mutant *nda3-TB101* (Yamamoto 1980; Minagawa et al. 2021), which conferred resistance against benzimidazole derivatives such as TBZ, MBC (carbendazim) and nocodazole, known as major anti-microtubule drugs. Drugs that affect viability of the MDR-sup *nda3-TB101* strain would be expected as 'new' drugs that target tubulin in a different manner from benzimidazoles. Using the strains, we selected drugs that substantially reduced viability of the *alp14*Δ (an ortholog of Dis1/TOG/XMAP215) and *bub1*Δ (a component of the spindle assembly checkpoint) mutants. Drugs that potentially target microtubules are supposed to reduce viability of those mutants more severely than that of WT. Isolated 31 candidate chemicals were then finally tested for a visual screen with *S. pombe* cells expressing GFP-tubulin. We discuss the efficiency and power of chemical genetic screens using yeast cells to search for novel drugs.

## TOR HD – REVEALING TOR REGULATION THROUGH CRYOEM

Robbie Joseph Loewith

University of Geneva, Dept. of Molecular and Cellular Biology, Geneva, Switzerland

The Loewith group focuses primarily on understanding how growth is controlled by the two Target Of Rapamycin Complexes, using the model eukaryote Saccharomyces cerevisiae where TOR was originally discovered. It has long been appreciated that TOR Complex 1 (TORC1) responds primarily to nutrient cues, in turn, regulating macromolecule synthesis and turnover and thus cell mass/volume. In contrast, we have recently discovered that TORC2 responds primarily to physical perturbations of the plasma membrane, in turn, regulating turgor pressure, lipid biosynthesis, and bilayer asymmetry and thus cell surface area. Although yeast has been traditionally exploited for its facile genetics, we have recently extended our studies to include structure determinations of the signaling nodes upstream of TORC1 and TORC2. In this talk I will highlight some of our recent discoveries including 1) the regulation of TORC1 by the SEA Complex and 2) how membrane perturbations generate conformational changes in eisosomes to influence TORC2 activity.

# A MULTISITE PHOSPHORYLATION SYSTEM CONTROLLING THE DECISION BETWEEN CELL DIFFERENTIATION AND PROLIFERATION

Mart Loog

University of Tartu, Institute of Technology, Tartu, Estonia

At the cell fate decision points, different signals are processed into alternative outputs. We present a mechanism regulating the decision between differentiation and proliferation in S.cerevisiae. The differentiation via the mating is triggered in G1 by the pheromone pathway leading to phosphorylation of Far1 by MAP kinase Fus3. Phosphorylated Far1 inhibits the cyclin-dependent kinase (CDK) to prevent the cell cycle. Alternatively, the CDK switches off the inhibition until the next G1 by also phosphorylating Far1, leading to its degradation. The CDK and MAPK signals with similar specificity for S/TP motifs are differentially processed via competing diversionary pathways on Far1 by phospho-adaptor Cks1 and short linear docking motifs (SLiMs) to prevent cross-circuiting. We also demonstrate synthetic logic gates built based on multisite phosphorylation modules of Far1.

# A GPI ANCHOR-INDUCED LIPID ORDER CHANGE TRIGGERS A PLASMA MEMBRANE STRESS RESPONSE

Li Chen, David K Banfield

The Hong Kong University of Science and Technology, Division of Life Science, Kowloon, Hong Kong

The modification of glycosylphosphatidylinositol (GPI) anchors is a complex process that plays a critical role in presenting functionally diverse proteins on the surface of eukaryotic cells. The GPI anchor provides the phosphatidylinositol group, enabling the protein to associate with the lipid microdomain - a subset of the liquid-ordered phase (Lo) of the biological membrane. This microdomain is essential for membrane trafficking, protein sorting, and signal transduction, typically in a clathrin-independent manner. The GPI anchor carbohydrate linker contains three mannose molecules which undergo precise ethanolamine phosphate (EtNP) remodeling, a requirement for GPI anchor biosynthesis and trafficking.

The biogenesis and remodeling of GPI anchors is multifaceted and many of the modifications are in the strict sense nonessential. Indeed, removal of EtNP from Man2 of GPI-anchored proteins (GPI-APs) was previously considered nonessential despite its evolutionary conservation. However, our recent research has revealed that this remodeling event is crucial for yeast viability. Impairing the removal of EtNP from Man2 results in the arrest of cell growth at the G2/M stage, but interestingly this growth arrest can be alleviated by disrupting activation of the spindle assembly checkpoint (SAC). However, if such unremodeled GPI-APs are continuously produced, the cell is no longer rescued from cell cycle arrest by deleting SAC genes, suggesting that the presence of EtNP on Man2 has more severe consequences than initially anticipated.

In this study, we demonstrate that GPI-APs bearing EtNP on Man2 are delivered to the cell surface but fail to be properly further processed. Such GPI-APs reduce their association with detergent-resistant membranes (DRMs) and cause an increase in the liquid-disordered phase (Ld) on the plasma membrane - which is considered a trigger for endocytosis. Indeed, the presence of Man2 unremodeled GPI-APs on the plasma membrane results in enhanced clathrin-dependent endocytosis. Endocytosed proteins are initially ubiquitinated and are thereafter delivered to the vacuole for degradation via the multivesicular body (MVB). These findings lead us to propose a stress response model in which proteins that residue outside the lipid microdomain are endocytosed to re-establish balance to the Lo/Ld phase on the plasma membrane.

# THE ROLE OF HISTONE H3 LYSINE 4 METHYLATION IN MAINTAINING REDOX HOMEOSTASIS UNDER ENDOPLASMIC RETICULUM STRESS

Chia-Ling Hsu, Wei-Hsun Hsiao, Tzu-Hsin Ku, Cheng-Fu Kao

Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

Methylation of histone H3 lysine 4 (H3K4me) has been long known to be associated with active gene expression. Despite its positive correlation with transcription, the underlying functional roles of H3K4me in transcription remain controversial; the loss of H3K4me has only a minor impact on global transcription. Over the past decade, reports on the H3K4me regulators mutated in various neurodevelopmental and neurological disorders, including intellectual disabilities, autism spectrum disorders, schizophrenia, and Alzheimer's disease, have been explored, highlighting the importance of H3K4me regulation in the homeostasis of the central nervous system. However, a lack of understanding of how H3K4me modulates neuronal function complicates the elucidation of its specific roles in this context. In this study, we report our finding that Set1, the only H3K4 methyltransferase in budding yeast, modulates the redox status of the endoplasmic reticulum (ER), potentially offering a new perspective on this issue. Specifically, we first discovered that the loss of Set1 or H3K4me genetically interacted with riboflavin kinase (Fmn1 in yeast), the rate-limiting enzyme in the synthesis of FAD. FAD is an essential cofactor for many enzymes, including ER oxidase (Ero1). Ero1 plays a pivotal role in disulfide bond formation (oxidative folding) in the ER, where it is maintained in an oxidizing environment to support oxidative folding. We subsequently discovered that the ablation of Set1 or H3K4me restored the viability of a loss-of-function version of Ero1. Mechanistically, the loss of Set1 or H3K4me reduced the ER stress caused by Ero1-deficiency by promoting oxidative folding, indicating that ER-stressed cells were more prone to an oxidized state due to the loss of H3K4me. Taken together, our data suggest for the first time that H3K4me plays a role in maintaining cellular ER-redox homeostasis, particularly under stress conditions, shedding new light on previously unknown molecular mechanisms of neurodevelopmental and neurodegenerative disorders.

# PIB2 IS A CYSTEINE SENSOR INVOLVED IN TORC1 ACTIVATION

Qingzhong Zeng[1], Yasuhiro Araki[2], Takeshi Noda[1,2]

[1]Osaka University, Graduate School of Biosciences, Osaka, Japan, [2]Osaka University, Graduate School of Dentistry, Osaka, Japan

TORC1 is a master regulator that monitors the availability of various amino acids to promote cell growth in Saccharomyces cerevisiae. It is activated via two distinct upstream pathways, the Gtr pathway, which corresponds to mammalian Rag, and the Pib2 pathway. This study showed that Ser3 was phosphorylated exclusively in a Pib2-dependent manner. Using Ser3 as an indicator of TORC1 activity, together with the established TORC1 substrate Sch9, we investigated which pathways were employed by individual amino acids. Different amino acids exhibited different dependencies on the Gtr and Pib2 pathways. Cysteine was most dependent on the Pib2 pathway, and increased the interaction between TORC1 and Pib2 in vivo and in vitro. Moreover, cysteine directly bound to Pib2 via W632 and F635, two critical residues in the T(ail) motif that are necessary to activate TORC1. These results indicate that Pib2 functions as a sensor for cysteine in TORC1 regulation.

# TRANSLATION FACTOR eIF5A IS THE SENSOR AND EFFECTOR FOR AUTOREGULATION OF CELLULAR POLYAMINES

Thomas E Dever, Ivaylo P Ivanov, Byung-Sik Shin, Arya Vindu, Chune Cao, Joo-Ran Kim

National Institutes of Health, NICHD, Bethesda, MD

Alterations in polyamine levels affects many cellular processes including translation, transcription, and ion channel function. An essential function of the polyamine spermidine is the post-translational formation of the amino acid hypusine on the translation factor eIF5A. The hypusine modification is essential for eIF5A stimulation of translation elongation and termination. Cells maintain polyamine homeostasis through control of polyamine synthesis and uptake; however, the polyamine sensor, transporter, and mechanism of regulation have been unclear. In a recent screen for mRNAs whose translation is under polyamine control, we identified the *HOL1* mRNA and showed that the Hol1 protein is the high-affinity polyamine transporter in yeast. The translational regulation of *HOL1* is dependent on a conserved upstream open reading frame (uORF) in the *HOL1* mRNA leader and we showed that polyamine inhibition of eIF5A function in translation termination on a conserved Pro-Ser-stop motif in the uORF controls *HOL1* mRNA translation. We now show that a similar mechanism controls polyamine synthesis.

The first step in polyamine synthesis is the conversion of ornithine to putrescine, catalyzed by the *SPE1*-encoded enzyme ornithine decarboxylase (ODC). The protein antizyme, encoded by *OAZ1*, binds to and inhibits ODC catalytic activity and triggers ubiquitin-independent turnover of ODC. Polyamine control of ODC is mediated indirectly by regulated synthesis of Oaz1. Polyamine-induced +1 ribosomal frameshifting on the *OAZ1* mRNA enables production of full-length functional Oaz1. Using in vivo reporter assays, we show that depletion of eIF5A mimics high polyamines and promotes frameshifting on the *OAZ1* mRNA. In addition, using an in vitro reconstituted translation assay system, we show that high polyamines inhibit termination and induce frameshifting on the *OAZ1* shift site and that these effects are suppressed by increasing eIF5A levels, indicating that polyamines competitively inhibit eIF5A function to control frameshifting.

In related studies in mammalian cells, we show that inhibition of eIF5A hypusination mimics high polyamines to inhibit translation termination and promote frameshifting on the *OAZ1* mRNA. Moreover, inhibition of hypusination also mimics high polyamines by inhibiting translation elongation and termination on conserved regulatory uORFs on the *AMD1* mRNA encoding S-adenosylmethionine decarboxylase, required for higher order polyamine synthesis, and on the *AZIN1* mRNA encoding a negative regulator of antizyme.

Taken together, our results demonstrate that polyamines feedback regulate their synthesis and uptake by modulating the activity of eIF5A in translation elongation and termination.

# OFF-TARGET REPRESSION BY GENOMIC CASSETTE INSERTION MASKS TRUE MUTANT PHENOTYPES

Emily Powers, Charlene Chan, Gloria Brar

UC Berkeley, Molecular and Cell Biology, Berkeley, CA

Targeted selection-based genome-editing approaches have enabled many fundamental discoveries and are used routinely with high precision. We found, however, that replacement of *DBP1*, the gene encoding an RNA helicase, with a common selection cassette in budding yeast led to reduced expression and function for the adjacent gene, *MRP51*, despite all MRP51 coding and regulatory sequences remaining intact. Cassette-induced repression of *MRP51* drove all mutant phenotypes detected in cells deleted for *DBP1*. This behavior resembled the 'neighboring gene effect' (NGE), a phenomenon of unknown mechanism whereby cassette insertion at one locus reduces the expression of a neighboring gene. We leveraged strong off-target mutant phenotypes resulting from cassette replacement of *DBP1* to provide mechanistic insight into the NGE, and to investigate the true cellular function of the Dbp1 helicase, both of which we will present.

Regarding the mechanism of off-target repression, the inherent bidirectionality of promoters, including those in expression cassettes, drives a divergent transcript that represses *MRP51* through combined transcriptional interference and translational repression mediated by production of a long undecoded transcript isoform (LUTI). Divergent transcript production driving this off-target effect is general to yeast expression cassettes and occurs ubiquitously with insertion. Despite this, off-target effects are often naturally prevented by local sequence features, such as those that terminate divergent transcripts between the site of cassette insertion and the neighboring gene. Thus, cassette-induced off-target effects can be eliminated by the insertion of transcription terminator sequences into the cassette, flanking the promoter. Because the driving features of this off-target effect are broadly conserved, our study suggests it should be considered in the design and interpretation of experiments using integrated expression cassettes in other eukaryotic systems, including human cells.

# IDENTIFICATION OF NOVEL QUALITY CONTROL FACTORS AT THE TOM COMPLEX

Arushi Gupta, Jiyao Song, Jeannine Engelke, Swadha Mishra, Fabian den Brave, Thomas Becker

University of Bonn, Biochemistry, Bonn, Germany

Mitochondria perform crucial functions for cell survival. In order to fulfil these functions, mitochondria contain about 1,000-1,500 proteins. About 99% of the mitochondrial proteins are synthesized as precursors on cytosolic ribosomes and imported into mitochondria via sophisticated import machineries. The translocase of the outer membrane complex (TOM complex) forms the entry gate for most of the mitochondrial precursor proteins. Defects in protein import via the TOM complex lead to accumulation of mitochondrial precursor proteins, which in turn causes massive proteotoxic stress. Consequently, defects in protein import have been linked to various neurological diseases.

To ensure that mitochondria function correctly, a plethora of quality control factors govern mitochondrial protein import at various steps, thereby ensuring proper protein import into mitochondria. In our research, we study quality control factors that monitor the TOM complex and prevent clogging of the protein translocon. Our lab identified the mitochondrial protein translocation associated degradation (mitoTAD) pathway that continuously removes arrested precursor proteins from the TOM complex to regenerate the translocase for protein import. Here, mitochondrially localized Ubx2 functions as the central component of the mitoTAD pathway by recruiting the AAA-ATPase Cdc48 to the TOM complex and facilitating the removal of precursor proteins that are arrested at the import channel. We now have identified a novel factor that binds to the TOM complex and functions in proteasome-dependent quality control of mitochondrial precursor proteins. Genetic and biochemical analysis reveals that this novel component functions independently of the mitoTAD pathway. Thus, our work implies the existence of multiple pathways acting in mitochondrial protein import quality control.

# ACIDIC GROWTH CONDITIONS STABILIZE THE RIBOSOMAL RNA GENE CLUSTER AND EXTEND LIFESPAN

Yo Hasegawa[1,2], Hiroyuki Ooka[1,2], Tsuyoshi Wakatsuki[1,3], Mariko Sasaki[1,2,5], Ayumi Yamamoto[4], Takehiko Kobayashi[1,2,3]

[1]The University of Tokyo, Institute for Quantitative Biosciences, Tokyo, Japan, [2]The University of Tokyo, Department of Biological Sciences, Tokyo, Japan, [3]Tokyo Institute of Technology, Department of Life Science and Technology, Tokyo, Japan, [4]Hachinohe College, Department of Industrial System Engineering, Hachinohe, Japan, [5]Current address, National Institute of Genetics, Mishima, Japan

Throughout the course of evolution, cells have undergone a progressive increase in size, accompanied by a concomitant augmentation in the complexity of cellular functions. These transformative shifts have led to an escalated demand for ribosomes within cells. Consequently, eukaryotic cells harbor extensive arrays of ribosomal RNA genes (rDNA) organized in tandem repeats on their chromosomes. Nevertheless, this repetitive structure triggers intragenic recombination among the repeats, resulting in the loss of gene copies, particularly notable in budding yeast. To counteract the depletion of these copies, the rDNA has evolved a gene amplification mechanism. As a result, the rDNA emerges as one of the most vulnerable regions with respect to alterations in the copy number of repeats, thus impacting its stability.

Blackcurrant (*Ribes nigrum* L.) is a traditional fruit with a rich history of use in the creation of juices, jams, and liqueurs. Blackcurrant extract is known to relieve cells from DNA damage caused by hydrogen peroxide ($H_2O_2$), methyl methane sulfonate (MMS), and ultraviolet (UV) radiation. We found that blackcurrant extract (BCE) plays a role in stabilizing the ribosomal DNA (rDNA) by repressing non-coding transcription within the intergenic spacer (IGS), consequently leading to an extended lifespan in budding yeast. Notably, manipulation of the growth medium's acidity to approximately pH 4.5 through the addition of HCl has shown to enhance rDNA stability and increase longevity. We identified *RPD3* as the gene responsible for this change, which was mediated by the RPD3L histone deacetylase complex. In mammals, where inflammatory sites in tissues exhibit acidity, a similar regulatory mechanism in DNA maintenance may exist to avert the potential onset of genome instability and subsequent cancer formation.

# BIOSYNTHESIS AND BIOPRODUCTION OF COENZYME Q IN YEASTS

Makoto Kawamukai

Shimane University, Life and Environmental Sciences, Matsue, Japan

Coenzyme Q (CoQ) is an essential component of the electron transport system in aerobic organisms. CoQ consists of a benzoquinone ring and a hydrophobic isoprenoid side chain with a certain number of isoprene units in an all-trans configuration. A CoQ-producing organism produces one type of CoQ as a main product, which is classified according to the length of the isoprenoid side chain. For example, *Homo sapiens* and *Schizosaccharomyces pombe* predominantly produce CoQ10 with 10 isoprene units, whereas *Arabidopsis thaliana* produce CoQ9, and *Saccharomyces cerevisiae* produces CoQ6 [1].
*S. japonicus* is an unique yeast which produce a very low levels of CoQ10, although all necessary genes for CoQ synthesis have been identified in its genome. *S. japonicus* had very low levels of oxygen consumption and was essentially respiration defective. *S. japonicus* grows well on minimal medium during anaerobic culture, indicating that it acquires sufficient energy by fermentation. S. japonicus produces comparable levels of ethanol under both normal and elevated temperature (42 °C) conditions, at which S. pombe is not able to grow [2].
CoQ10 is especially valuable as a food supplement. Therefore bioproduction of CoQ10 in fission yeast is an important aspect for commercial purpose. We found that CoQ10 level was higher in stationary phase than in log phase, and that it increased when the cells were grown in a low concentration of glucose, in maltose, or in glycerol/ethanol medium. Because glucose signaling is mediated by cAMP, we evaluated the involvement of this pathway in CoQ biosynthesis. Loss of Pka1, the catalytic subunit of cAMP-dependent protein kinase, increased production of CoQ10, whereas loss of the regulatory subunit Cgs1 decreased production. Manipulation of other components of the cAMP-signaling pathway affected CoQ10 production in a consistent manner [3].
Because the CoQ biosynthetic pathway has not been fully elucidated, we investigated CoQ10 production in 400 S. pombe gene-deleted strains in which individual mitochondrial proteins were lost. We found that deletion of *coq11* and a novel gene designated *coq12* lowered CoQ levels to 2% of that of the wild-type strain. Addition of PHB or p-hydroxybenzaldehyde restored the CoQ content and growth, and lowered $H_2S$ production of the Δ*coq12* strain. Purified Coq12 protein from S. pombe displayed $NAD^+$ reductase activity when incubated with ethanol-extracted substrate of S. pombe. Analysis of Coq12-interacting proteins by liquid chromatography-tandem mass spectrometry (LC-MS/MS) revealed interactions with other Coq proteins, suggesting formation of a complex [4].

[1] Kawamukai Biosci. Biotechnol. Biochem. 2016
[2] Kaino et al., Biosci. Biotechnol. Biochem. 2019
[3] Nishida et al., Appl. Microbiol. Biotechnol. 2019
[4] Nishida et al., J. Biol Chem. 2023

# DECOUPLING OF NUCLEAR VOLUME AND SURFACE AREA DURING MATING

Ellen Morgan, Amanda Yeo, Val Meleshkevich, Orna Cohen-Fix

National Institutes of Health, The Laboratory of Biochemistry and Genetics, NIDDK, Bethesda, MD

Nuclei of most cells are round or oval, but some cell types deviate from this norm. One such example is during budding yeast mating, when nuclei appear elongated in response to mating pheromone (Stone et al, Curr. Biol. 2000). Changes in nuclear morphology are also observed in disease and during development, but the processes that drive and regulate these shape changes are largely unknown. Nuclear elongation during mating was also shown to be under the control of the Ste12 transcription factor, the master regulator of the mating response. Since Ste12 has a finite number of targets, the process of nuclear elongation in response to mating pheromone (NERPH) could serve as a tractable system to understand how cells drive and regulate changes to nuclear morphology.

To get a better idea of the types of genes that may be involved in NERPH, we first characterized its requirements. In principle, a nucleus can transition from round to elongated in one of two ways: it can either lose volume or it can gain surface area. To examine which possibility applies to NERPH, cells were synchronized in G1 by cyclin depletion, and then exposed to mating pheromone (alpha factor). First, we observed that NERPH is dependent on fatty acid synthesis, suggesting that it requires de novo synthesis of phospholipids. Second, exposure of cells to mating pheromone led to increase in both nuclear volume and surface area compared to untreated control cells. Interestingly, under these conditions, cell size was the same in the presence or absence of pheromone, suggesting that mating pheromone instructs the cell to direct more resources to the nucleus. Finally, the rate of nuclear surface area increase exceeded that of volume increase, causing nuclei to elongate. Thus, NERPH is a result of preferential increase in nuclear surface area.

The reason for NERPH is not known. To explore this, mutants defective in NERPH would be useful. We thus screened >180 mutants deleted in known Ste12 targets (Roberts et al, Science 2000). Two mutants were partially defective in NERPH: deletion of the Kar4 transcription factor, and deletion of Prm3, which is required for nuclear fusion during mating. Kar4 is upstream of Prm3, and indeed the *kar4*Δ *prm3*Δ double mutant had the same partial defect in NERPH as each of the single mutants, suggesting that we are missing one or more targets that are downstream of Ste12 but not Kar4. Moreover, we observed that the role of Prm3 in NERPH is independent of its function in nuclear fusion. To identify the missing NERPH pathway that acts in parallel to the Kar4-Prm3 axis, we carried out RNA seq analyses of wild type, *ste12*Δ and *kar4*Δ with and without pheromone and using synchronized cells, as previous analyses were done using microarrays and starting with asynchronous cultures. The results of this analysis, as well as other properties related to NERPH, will be discussed.

# 3, 3'-DIINDOLYLMETHANE (DIM) AFFECTS THE LOCALIZATION OF PROTEINS IN BOTH NUCLEAR ENVELOPE AND NUCLEAR ER IN FISSION YEAST

Hideto Nagai[1], Kaiyu Wang[2], Hyekyung Seol[2], Masaru Ueno[1,2]

[1]Hiroshima University, Cluster 3, School of Engineering, Higashi-Hiroshima, Japan, [2]Hiroshima University, Graduate School of Integrated Sciences for Life, Higashi-Hiroshima, Japan

3, 3'-Diindolylmethane (DIM) is a compound derived from the digestion of indole-3-carbinol, found in cruciferous vegetables. DIM is a potent anti-cancer drug. However, the molecular target of DIM is not fully understood. We reported that DIM induces apoptosis and autophagy, and disrupts nuclear envelope in fission yeast [1]. However, mechanism of the nuclear envelope disruption remains unclear. Here, we show that DIM makes hole in nuclear envelope. Localization of both inner nuclear membrane protein Ish1 and ER protein Ost4 is affected by DIM addition. One of the ESCRTIII component Cmp7 is recruited to nuclear envelope in the presence of DIM. Moreover, mutation in cmp7, ned1 (the phosphatidic acid phosphatase of the lipin family), pik3 (phosphatidylinositol 3-kinase) and ksg1 (3-phosphoinositide-dependent protein kinase) made cells sensitive to DIM. These results suggest that DIM affects protein and/or lipid composition in nuclear envelope and nuclear ER. We also isolated the mutants that are less DIM sensitive than wild-type strain. Gene mutations responsible for DIM resistance were speculated by next generation sequencing. Re-introduction of the candidate mutation to wild type strain revealed that tsf1 mutation make cell resistant to DIM. Tsf1 is a mitochondrial translation elongation factor. We will discuss about possible mechanism of the DIM resistant by tsf1 mutation.

References

[1]. Emami P., Ueno M. PLoS One, 2021, 16(12):e0255758.

# WHY ARE BUDDING YEAST CHROMOSOMES THINNER THAN THOSE IN FISSION YEAST?

Yasu Kakui[1], Minzhe Tang[2], Christopher Barrington[2], Hideki Kanizawa[3], Ken-Ichi Noma[3], Frank Uhlmann[2]

[1]Waseda University, Waseda Institute for Advanced Study, Tokyo, Japan, [2]The Francis Crick Institute, Chromosome Segregation Laboratory, London, United Kingdom, [3]Hokkaido University, Institute for Genetic Medicine, Sapporo, Japan

Mitotic chromosomes in different organisms adopt various dimensions, the determinants for which are poorly understood. We start by comparing fission yeast and budding yeast, which harbor similarly sized genomes distributed amongst 3 or 16 chromosomes, respectively. Superresolution and genomic approaches reveal a species-specific chromosome width determinant, which we find correlates with the spacing intervals between binding sites of the chromosomal condensin complex. Unexpectedly, within each species, longer chromosome arms are always thicker, a universal relationship that also applies to human chromosomes. We use this information to explore molecular models for how condensin gives mitotic chromosomes their shape.

# DNA REPAIR PROTEINS AT THE R LOOP MODULATE THE LEVEL OF GENE TRANSCRIPTION

Hiroshi Masumoto[1], Hideki Muto[1], Yohei Kurosaki[2], Koichiro Yano[3], Hironori Niki[3]

[1]Biomedical Research Support Center, Nagasaki University School of Medicine, Nagasaki, Japan, [2]National Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University, Nagasaki, Japan, [3]Micribial Physiology Laboratory, Department of Gene Function and Phenomics, National Institute of Genetics, Mishima, Japan

The R-loop structure consists of DNA:RNA duplexes and single-stranded DNA and is mainly formed during gene transcription. The R-loop serves as a platform for transcription factors and other proteins with different functions. DNA damage repair factors also assemble in the R-loop to repair possible DNA damage on the R-loop. In this study, we show that DNA repair proteins are involved in the repression of gene transcription.

Esc2 and Rad57, which are both involved in DNA homologous recombination repair, were isolated as factors involved in the silencing within the Ty1 retrotransposon coding region in budding yeast. Chromatin immunoprecipitation sequencing (ChIP-seq) showed that Esc2 and Rad57 localise to a group of actively transcribed genes such as house-keeping genes. The fact that the localisation of Esc2 and Rad57 is restricted to the region from the gene body to the 3' UTR, and not to the promoter region, and that the amount of binding of both factors to this region is dependent on promoter activity, suggests that Esc2 and Rad57 bind to the R-loop. Furthermore, the *esc2Δ rad57Δ* double deletion strain increased transcript levels of house-keeping genes, suggesting that Esc2 and Rad57 repress transcription of genes located in the R-loop.

To investigate why transcription is increased in the *esc2Δ rad57Δ* strain, we compared the amount of transcription complexes bound to gene regions in the wild-type strain and the esc2Δ rad57Δ strain. We found that RNA polymerase II (or Pol II), which is responsible for mRNA and snRNA synthesis, increased on housekeeping genes in the *esc2Δ rad57Δ* strain, but decreased on transiently up-regulated genes such as cell cycle control genes. *esc2Δ rad57Δ* strain not only consumed less glucose and proliferated at a slower rate than the wild-type strain, but also reduced its cell lifespan. Thus, Esc2 and Rad57 assemble on the R-loop and are responsible not only for fixing DNA damage but also for regulating gene transcription. This transcriptional regulation is important for cell proliferation and lifespan.

# ACETYLATION OF REC8 COHESIN COMPLEXES REGULATES MONO-ORIENTATION OF KINETOCHORES

Ziqiang Li, Yu Liu, Yoshinori Watanabe

Jiangnan University, Science Center for Future Foods, Wuxi, China

In proliferating cells, sister chromatid cohesion is established in S phase and maintained until metaphase, and required for bipolar attachment of chromosomes to spindles and thereby equational division in mitosis. In meiosis, however, sister kinetochores attach to microtubules emanating from the same pole (mono-polar attachment) while homologous kinetochores attach to the opposite poles, leading to reductional division. For establishing sister chromatid cohesion, acetyltransferase Eco1 (Eso1 in fission yeast) plays a key role by acetylating the cohesin complex, especially at two lysine residues in Smc3 (K105 and K106 in Psm3 in fission yeast). Although Eso1 also contributes to the establishment of mono-orientation of kinetochores in meiosis, underlying molecular mechanisms remain elusive (ref. 1). Here, we analyze centromere-associated Rec8 cohesin complexes and identify meiosis-specific acetylation at the conserved residue K1013 in Psm3. Psm3-K1013 acetylation occurs largely depending on the meiotic kinetochore regulator meikin (Moa1). Our molecular genetic analyses indicate that in combination with canonical acetylation at Psm3-K105 and K106, Psm3-K1013 acetylation plays a crucial role in establishing mono-orientation in meiosis.

1) Kagami et al. Acetylation regulates monopolar attachment at multiple levels during meiosis I in fission yeast. EMBO rep. 12, 1189-1195 (2011)

**NOTES**

**NOTES**

**NOTES**

**NOTES**

## Participant List

Ms. Fara Difka Afdilla
Hiroshima University
m235743@hiroshima-u.ac.jp

Ms. Ryoko Ando
Kyoto Institute of Technology
ryokoicha.1119@gmail.com

Dr. Brenda J Andrews
University of Toronto
brenda.andrews@utoronto.ca

Dr. Charlie Boone
University of Toronto
charlie.boone@utoronto.ca

Dr. Gloria Brar
University of California, Berkeley
gabrar@berkeley.edu

Dr. Yu Chan
Peking University
yu_chan@stu.pku.edu.cn

Dr. Fred Chang
University of California, San Francisco
fred.chang@ucsf.edu

Dr. Li CHEN
The Hong Kong University of Science and Technology
lichen@ust.hk

Dr. Ramveer Choudhary
IFOM ETS
ramveer.choudhary@ifom.eu

Dr. Orna Cohen-Fix
NIDDK-NIH
ornac@niddk.nih.gov

Dr. Tom Dever
National Institutes of Health
thomas.dever@nih.gov

Dr. Li-lin Du
National Institute of Biological Sciences, Beijing
dulilin@nibs.ac.cn

Dr. Marco F. Foiani
IFOM The FIRC Institute of Molecular Oncology
marco.foiani@ifom.eu

Ms. Yuri Fujita
Okayama University
pkry58y3@s.okayama-u.ac.jp

Ms. Arushi Gupta
University of Bonn
agupta@uni-bonn.de

Ms. Eugenia HADDAD
IFOM ETS
eugenia.haddad@ifom.eu

Dr. Sachiko Hayashi
University of Hyogo
shayashi@sci.u-hyogo.ac.jp

Prof. Xiangwei He
Zhejiang University
xhe@zju.edu.cn

Ms. Sandra Heinen
University Bonn
sheinen@uni-bonn.de

Ms. Kar Ling Hoh
Temasek Life Sciences Laboratory
karling@tll.org.sg

Mr. Ira J Horecka
University of Toronto
ira.horecka@mail.utoronto.ca

Mr. Zaifeng Hu
Peking University
zephyr_hu@pku.edu.cn

Mr. Woosang Hwang
Hiroshima university
d236703@hiroshima-u.ac.jp

Dr. Atsuko Ikeda
Hiroshima University
atsukoikeda@hiroshima-u.ac.jp

Dr. Hiroshi Iwasaki
Tokyo Institute of Technology
hiwasaki@bio.titech.ac.jp

Dr. SHINGO IZAWA
Kyoto Institute of Technology
thioredoxin@kit.ac.jp

Mr. Eunhong Jang
Gist
eunhong0714@gist.ac.kr

Ms. Mengxue JIANG
The University of HONG KONG
u3008272@connect.hku.hk

Ms. Jomkwan Jumpathong
Tottori university
d17a3105@matsu.shimane-u.ac.jp

Dr. Youngsoo Jun
Gwangju Institute of Science and Technology
junys@gist.ac.kr

Dr. Yasutaka Kakui
Waseda University
yasukakui@aoni.waseda.jp

Dr. Yutaka Kanoh
Tokyo Metropolitan Institute of Medical Science
kanou-yt@igakuken.or.jp

Dr. Cheng-Fu Kao
Academia Sinica
ckao@gate.sinica.edu.tw

Dr. Makoto Kawamukai
Shimane University
kawamuka@life.shimane-u.ac.jp

Ms. yuseon Kim
Gist
dbtjs0202@gm.gist.ac.kr

Mr. Minsuk Kim
Gist
ishs161107@gm.gist.ac.kr

Prof. Yoko Kimura
Shizuoka University
kimura.yoko@shizuoka.ac.jp

Dr. Masao Kishida
Osaka Metropolitan University
masyksd@omu.ac.jp

Ms. NAHO KO
Gist
konaho4048@gist.ac.kr

Dr. Takehiko Kobayashi
The University of Tokyo
tako2015@iqb.u-tokyo.ac.jp

Dr. Daochun Kong
Peking University
kongdc@pku.edu.cn

Dr. Keiko Kono
Okinawa Institute of Science and Technology Graduate University
KEIKO.KONO@OIST.JP

Mr. Zhijian Kuang
HongKong University
u3007569@connect.hku.hk

Ms. Ruchika Kumari
JNCASR
ruchika231095@gmail.com

Ms. Lingyan Li
Peking University
lingyan0101@163.com

Ms. Bingyi Li
Tokyo Metropolitan Institute of Medical Science
libingyi25@outlook.com

Dr. Qing Li
Peking University
li.qing@pku.edu.cn

Dr. Yi-Chen Lo
National Taiwan University
loyichen@ntu.edu.tw

Dr. Robbie Loewith
University of Geneva
Robbie.Loewith@unige.ch

Dr. Mart Loog
University of Tartu
Mart.Loog@ut.ee

Mr. Aoi Makita
Tohoku University
aoi.makita.t7@dc.tohoku.ac.jp

Ms. Xin Man
Chinese Academy of Sciences
zhoulab@sibcb.ac.cn

Dr. Hisao Masai
Tokyo Metropolitan Institute of Medical Science
masai-hs@igakuken.or.jp

Dr. Hiroshi Masumoto
NAGASAKI UNIVERSITY
himasumo@nagasaki-u.ac.jp

Ms. Ruishuang Meng
Peking University
1901110501@pku.edu.cn

Ms. AKIKO MINAGAWA
Tokyo Metropolitan Institute of Medical Science
minagawa-ak@igakuken.or.jp

Prof. Hisao Moriya
Okayama University
hisaom@okayama-u.ac.jp

Mr. Kongmin Na
Gist
skrhdals12@gm.gist.ac.kr

Dr. SHIRISHA NAGOTU
INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
snagotu@iitg.ac.in

Prof. Takuro Nakagawa
Osaka University
nakagawa.takuro.sci@osaka-u.ac.jp

Dr. Jun-ichi Nakayama
National Institute for Basic Biology
jnakayam@nibb.ac.jp

Dr. Shotaro Namba
Okayama University
plt713ad@s.okayama-u.ac.jp

Dr. Takeshi Noda
Osaka University
noda.takeshi.dent@osaka-u.ac.jp

Dr. Ken-ichi Noma
University of Oregon
noma@uoregon.edu

Dr. Yoshinori Ohsumi
Tokyo Institute of Technology
ohsumi.y.aa@m.titech.ac.jp

Dr. Koji Okamoto
Osaka University
okamoto.koji.fbs@osaka-u.ac.jp

Dr. Snezhana Oliferenko
King's College London
snezhka.oliferenko@crick.ac.uk

Dr. Lien Thi Kim Pham
RIKEN
lien.pham@riken.jp

Dr. Ivan Psakhye
IFOM - The AIRC Institute of Molecular Oncology
ivan.psakhye@ifom.eu

Dr. Dirk Remus
Memorial Sloan Kettering Cancer Center
RemusD@mskcc.org

Ms. Tanveera R Sarhadi
Indian Institute of Technology, Guwahati
tanveera.rounaque@iitg.ac.in

Dr. Mariko Sasaki
National Institute of Genetics
m_sasaki@nig.ac.jp

Dr. Masamitsu Sato
Waseda University
masasato@waseda.jp

Dr. Jiyao Song
University of Bonn
asong@uni-bonn.de

Ms. Xiaoyu Su
Peking University
suxiaoyu@pku.edu.cn

Prof. Qian-Quan Q Sun
University of Wyoming
neuron@uwyo.edu

Dr. Mitsuaki Tabuchi
Kagawa University
tabuchi.mitsuaki@kagawa-u.ac.jp

Mr. Jiashen Tang
HIROSHIMA UNIVERSITY
tangjiashen123@gmail.com

Dr. Junko Y Toshima
Tokyo University of Technology
toshimajk@stf.teu.ac.jp

Prof. Jiro Toshima
Tokyo University of Science
jtosiscb@rs.tus.ac.jp

Dr. Suchin Towa
Kyushu University
towa.suchin.979@m.kyushu-u.ac.jp

Dr. Hideo Tsubouchi
Tokyo Institute of Technology
htsubouchi@bio.titech.ac.jp

Dr. Masaru Ueno
Hiroshima University
scmueno@hiroshima-u.ac.jp

Dr. Frank Uhlmann
The Francis Crick Institute
frank.uhlmann@crick.ac.uk

Dr. Kaiyu Wang
Hiroshima university
m225708@hiroshima-u.ac.jp

Mr. Gengchao WANG
The University of Hong Kong
gc.wang@outlook.com

Dr. Yoshinori Watanabe
Jiangnan University
ywatanabe@jiangnan.edu.cn

Mr. Kosuke Yamazaki
Tokyo Metropolitan Institute of Medical Science
yamazaki-ks@igakuken.or.jp

Dr. Yoko Yashiroda
RIKEN Center for Sustainable Resource Science
ytyy@riken.jp

Ms. Tian-Tian Ye
Chinese Academy of Sciences
zhoulab@sibcb.ac.cn

Mr. Ziyun YE
The Hong Kong University of Science and Technology
zyeai@connect.ust.hk

Ms. Yujie Zhang
Peking University
15zhang@pku.edu.cn

Ms. Chunzhao Q Zhang
UNIVERSITY OF WYOMING
chunzhao@uwyo.edu

Dr. Dan Zhang
Temasek Life Science Laboratory
zhangdan@tll.org.sg

Mr. Wenshuo Zhang
Peking University
1801110536@pku.edu.cn

Mr. Zeqi Zheng
Peking University
1701111505@pku.edu.cn

Ms. Wanxin Zheng
Tokyo Metropolitan Institute of Medical Science
z13032856850@outlook.com

Dr. Jin-Qiu Zhou
Institute of Biochemistry and Cell Biology, CAS
jqzhou@sibcb.ac.cn

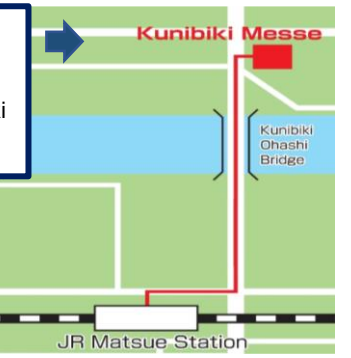
## CONVENTION CENTER

### Kunibiki Messe (Shimane Prefectural Convention Center)

The biggest convention center in Shimane prefecture, Kunibiki Messe, is located in the center of Matsue City. There are Exhibition hall (4,018 sqm), Multipurpose hall (686 sqm), International conference hall (510 sheets), and 19 meeting rooms.

**Free Wi-Fi** is available in building.

It takes only 7 minutes on foot from JR Matsue Station to Kunibiki Messe




KUNIBIKI MESSE
Address: 1-2-1 Gakuen Minami Matsue City, Shimane, JAPAN 690-0826
TEL +81+852-24-1111 FAX:+81+852-22-9219
E-mail: kunibiki@kunibikimesse.jp
URL: http://www.kunibikimesse.jp

# Kunibiki Messe Floor Plan