Exploration of exogenous bornaviruses and endogenous bornavirus-like elements

Bornaviruses are RNA viruses from the family Bornaviridae which had consisted of one genus Bornavirus with a single species Borna disease virus until 2008. However, recent several studies have revealed the presence of many novel bornaviruses in vertebrate species. Additionally, in 2010, we discovered sequences derived from bornaviruses in the genomes of mammals. These findings have expanded our knowledge on the genetic diversity bornaviruses, which are useful to understand the deep evolutionary history of RNA viruses and the co-evolution between RNA viruses and their hosts. Here I present our researches on exploration of exogenous bornaviruses and endogenous bornavirus-like elements as well as our recent findings about the paleovirology of bornaviruses and the co-evolution between bornaviruses and mammals.

Genomic basis for the global distribution of ultra-small bacteria

Filtration with a 0.2 \(\mu\)m filter is a common method for the removal of microorganisms from samples for use in scientific research and many other applications. However, several studies have shown that novel lineages can nonetheless remain in filtered environmental samples. Here, we report that ultra-small bacteria can pass through 0.2 \(\mu\)m filters. Environmental samples were three-fold filtered with 0.2 \(\mu\)m filter units, and each filtrate was incubated in a different type of growth medium. In total, nearly 50 strains were isolated. Phylogenetic analysis revealed that one actinobacterial strain belonged to the Luna2 lineage, which has a cosmopolitan distribution across inland freshwater ecosystems. This strain was characterized by ultramicro-sized cells (0.1 \(\mu\)m), even under eutrophic culture conditions, and an extremely small, streamlined genome of approximately 1.6 Mb. Such a reduction in genome size may be associated with lowering resource costs. Additionally, genomic annotation of this strain suggests that it undergoes rhodopsin-based photometabolism. Thus, our results indicate that unique, cultivable ultramicrobacteria are present in filtered samples.

Molecular analysis of the Xer/dif system for chromosome resolution in archaeon Thermoplasma acidophilum

Xer-mediated site-specific recombination plays a critical role in segregation of circular chromosome after DNA replication. The Xer recombinases resolve chromosome dimers to monomers at a specific DNA sequence, called dif. In this study, we identified two binding sites for a Xer from archaeon Thermoplasma acidophilum by ChIP-seq analysis. In vitro analysis further confirmed that one of two sites serves indeed as a dif site for XerA. Moreover, systematic mutational analysis provided further insights into how XerA recognizes the cleavage site and mediates site-specific recombination.

Acetic acid confer the plant drought tolerance

Drought severely impacts plant survivability. Here, we demonstrate that plants possess a survival strategy; acetate is synthesized through the dynamic metabolic flux conversion from glycolysis in response to drought and confers plant drought tolerance by stimulating the phytohormone jasmonate signaling pathway. Exogenous application of acetic acid successfully enhanced drought tolerance in monocots and dicots. Arabidopsis histone deacetylase HDA6 directly regulates drought tolerance as an initial ON/OFF switch of the whole acetate-mediated novel network. Our findings highlight this radically new survival strategy enabling plants to endure water deficit; with potential to serve as a powerful application to maintain crops under drought conditions.
Dynamics of DnaA-oriC complex for duplex unwinding and helicase loading to initiate DNA replication

In *Escherichia coli*, the initiator protein DnaA forms specific oligomers on the replication origin oriC. DnaA-oriC complex locally unwinds DNA in concert with the DNA-binding protein IHF, followed by loading of two replicative helicases loading onto the resultant single-stranded DNA. oriC consists of two functional regions: the duplex unwinding element (DUE) and its flanking DnaA-oligomerization region (DOR). The DOR includes at least 12 DnaA-binding sequences (DnaA boxes) and a single binding site of IHF. DUE unwinding occurs by a subcomplex formed on the left-half DOR including the IHF-binding site and DUE-flanking six DnaA boxes, while helicase loading requires both subcomplexes formed on the left- and right-halfs DOR. Here, roles of DnaA protomers on the each half DOR were examined in detail and functional structures for DUE unwinding and helicase loading complexes will be proposed.

Maintenance of unperturbed DNA replication by ubiquitylation of the replication sliding clamp

In many organisms, modification of the eukaryotic replication clamp PCNA (PCNA-Ub) was reported to regulate DNA damage tolerance pathways, facilitating translesion DNA synthesis and template switching events. The roles of these pathways were well established for tolerance of replication blocking lesions; however, PCNA-Ub was associated with progression of unperturbed replication in many organisms and it remains unclear if PCNA-Ub contributes to the intrinsic nature of DNA replication. In fission yeast, PCNA-Ub is primarily observed during unperturbed replication and the level of ubiquitylation is barely increased by DNA damage. Importantly, our experiments indicated that PCNA-Ub mediates filling of ssDNA gaps during unperturbed mid-late S phase. We reasoned that this effect is due to altered stability or processivity of the lagging strand DNA polymerase (Polβ). Consistent with this model, we observed that PCNA-Ub stabilises PCNA which is loaded onto DNA and consequently causes increased association of PCNA with Polβ. Based on these results, we argue that PCNA-Ub during unperturbed S phase contributes to not only DNA damage tolerance but also efficient genome replication.

Evolution of sex chromosomes: Why are they so pervasive?

When sex chromosomes emerge from a pair of autosomes, the Y chromosome mostly becomes deteriorated due to the accumulation of pseudogenes and transposable elements. Despite this potential disadvantage, many organisms have independently acquired the sex chromosomes during evolution. One of the approaches to tackle this mystery is to trace the degeneration process of the Y. However, tracing such degeneration is difficult, because the sex chromosomes are so old in many species that the Y degeneration is already completed in these species including model organisms like Drosophila melanogaster. Therefore, I have focused on another Drosophila species, D. miranda, whose sex chromosomes are ~1 million years old. Using a comparative genomics/transcriptomics approach, I so far found that 1) more than half of the genes on the Y in D. miranda are already pseudogenized, but 2) up-regulation of X-linked genes in males offsets the effect of pseudogenization on the Y. However, I also found that 3) not only the Y but also the X is under an accelerated pseudogenization. These findings indicate that having sex chromosomes is potentially more deleterious for organisms than previously thought.

Neuronal mechanisms of evolution of species-specific pheromone preference in Drosophila

Although animals generally acquire the species-specific mating preference in the speciation process, what neural mechanisms contribute to such evolutionary change remains unclear. Between Drosophila melanogaster and the sister species D. simulans, the preference to the melanogaster-specific female pheromone, 7,11-heptacosadiene (7,11-HD), is totally opposite; 7,11-HD increases courtship of D. melanogaster males, while dramatically inhibits that of D. simulans. To uncover what neural mechanisms underlying the evolutionary change of 7,11-HD preference, we focused on gustatory ppk25 neurons, which reportedly receive 7,11-HD and promote courtship in D. melanogaster.

To examine the hypothesis that ppk25 neurons are involved in the negative 7,11-HD preference in D. simulans, we silenced these neurons in F1 hybrid males of the focal two species, which were repelled by 7,11-HD as D. simulans males are. Contrary to our expectation, neural silencing of ppk25 neurons did not increased courtship to D. melanogaster females, suggesting that ppk25 neurons are not involved in the negative 7,11-HD preference in hybrids and D. simulans.


The molecular basis of distinct responses to nutrient balances between Drosophila generalist and specialist species

In nature, developing animals cope with changeable nutritional conditions. To unravel the underlying mechanisms, we are using comparative multi-omics approaches to examine dietary responses of two generalist species including D. melanogaster and three specialists that feed on specific fruits or flowers in nature. We tested the effects of isocaloric diets with increasing protein-to-carbohydrate ratios on developmental rate and time, levels of metabolites, and gene expression profiles. The larvae of the generalist species developed normally on all of the diets, whereas specialist larvae failed to develop on a high-carbohydrate diet. Whole-body RNA-seq highlighted generalist responsive genes that were up-regulated on a carbohydrate-rich diet in the generalists, but hardly up-regulated or even down-regulated in the specialists. We are using GC-MS analysis to characterize how the above changes in gene expression might impact the relevant metabolism. We will discuss our current assessment of roles of the generalist responsive genes in metabolic homeostasis and adaptation and our approaches to understand nutrient balances.


Toward understanding the mechanism of color pattern formation in Drosophila guttifera

Animals have a variety of color patterns, but the mechanisms which regulate color patterns are not well understood. We use a polka-dotted fruit fly, Drosophila guttifera, to unravel the mechanisms of color pattern formation. We previously discovered that the gene wingless is involved in the process of induction of pigmentation (Werner et al. 2010). Then we showed that cis-regulatory evolution was important for acquiring the color pattern (Koshikawa et al. 2015, Koshikawa 2015). However, factors which act downstream of wingless in this process have not been identified except for the melanin synthesis pathway gene yellow. We are trying to unravel the entire system of color pattern formation of D. guttifera by utilizing various approaches.

References
Introduction of newly-developed protein analysis using mass spectrometry

Mass spectrometry (MS) is a widely employed approach to identification and quantification of metabolites, proteins and various post-translational modifications. In this talk, I will introduce two MS approaches: Stable Isotope Labeling using Amino Acids in Cell culture (SILAC) and Proximity-dependent Biotin Identification (BIO-ID)

One approach is to identify a substrate modified by modifiers using SILAC. SILAC is a MS approach for quantification of protein, which amino acids are labeled with different stable isotopes. I will show the data to identify several glycolytic enzymes and ribosome proteins as substrates deacetylated by NAD+-dependent deacetylases from budding yeast cell proteins using SILAC.

Another approach is to identify an interacting proteins using BIO-ID. BIO-ID is a method to screen the proximal proteins biotinylated by biotin ligase fused with protein of interest within cells. I will show the data to identify candidate proteins interacted with NAD+-dependent deacetylases from budding yeast cell proteins using BIO-ID.

Proteomics strategies for quantitative and qualitative analysis using mass spectrometry in combination with stable isotope labeling technique

A verity of mass spectrometry-based experimental techniques for large-scale protein analyses has accelerated proteomics studies including quantification of protein expression profiles and dynamics, identification of post-translational modifications, and understanding protein-protein interactions. In particular, technical platforms using stable isotope labeling methods can be applied to not only comparing or measurement of protein expression levels but also assessment of the specificity for identified proteins and determination of the regulatory mechanism by which their expressions are modulated. Here we present two types of strategies incorporating stable isotope labeling procedures that allowed us to quantify protein copy number with high accuracy and Know changes in protein ages accompanied by aging process, both of which are originally developed in our group. Development of a unique strategy would promise advances of understanding of proteome at various aspects as well as an increase in analysis throughput and coverage over proteome.

Next generation sequencer as a tool of genome analysis in human with genetic/genomic diseases

Development of next-generation sequencer (NGS) allowed low-cost genomic sequencing that made a great advances of genomic studies in many species. Even in human, who have a large genome, NGS can analyses personal genome and can perform clinical sequences.

From 2008, NIH started a big project of clinical exome sequencing in U.S.A. The project has made a success now. In the UK, NHS and Welcom Trust organized a project of whole genome sequencing in 1,000 families of rare diseases from 2011. Then, one of the biggest projects, 10M-genome project by Genomics England is progressing. In addition, President Obama released 'Precision Medicine Initiative’ in January 2015. Genome analysis including whole genome analysis is a trend in the world, and one of the targets of such projects is rare disease (genetic disease).

In Japan, one genome project, Initiative on Rare and Undiagnosed Diseases in Pediatrics; IRUD-P has been launched in July 2015. One aims of the project is to reach a diagnosis in undiagnosed patients by NGS analysis. From July 2015 to April 2016, many consultations were reached and trio analyses gave a lot of results.

I present the outline and current results of the project first. Then, I show another value of the project, and show important points and pit falls on genome analysis by NGS.
The imaging of ER-Golgi and intra-Golgi protein transport

Eukaryotic cells have the elegant trafficking systems which deliver enormous numbers and kinds of proteins to their own destinations. One of the important trafficking system is membrane traffic, which delivers proteins between organelles by membrane-bounded vesicles and carrier intermediates. The basic mechanism of membrane traffic is conserved in all eukaryotic cells, from yeast to higher animals and plants. It also underlies the fundamental need for cells to maintain cellular homeostasis. Newly synthesized proteins in the ER are delivered to the cis face of the Golgi apparatus. They are processed and modified during the delivery from cis cisternae to trans cisternae of the Golgi and then they are selectively sorted to deliver to their final destination from trans cisternae. *S. cerevisiae* has unique Golgi cisternae, which are dispersed and moving in the cytoplasm. Today, I show the 4D imaging data about cargo protein delivery from the ER to the Golgi apparatus and between the Golgi cisternae.

Defining the importance of oxidative DNA damage repair system on genome integrity in mice

Spontaneous mutations arise from many different sources, such as DNA lesions induced by reactive oxygen species. Enzyme systems responsible repairing oxidative base damages have been found in a variety of organisms from bacteria to humans. To clarify the role of DNA repair systems in the mutation avoidance in mammals, we have been studying somatic mutations, tumor incidence and germline mutations by using oxidative DNA repair pathway-deficient mice. Increased rate of somatic mutation, predominantly G to T transversions, was observed in the *Mutyh* (a base excision repair enzyme which prevents 8-oxoguanine-induced mutations)-deficient mice, together with increased intestinal tumor incidence. To analyze germline effects of *Mth1*, *Ogg1* and *Mutyh* (genes responsible preventing 8-oxoguanine-induced mutation), triple gene knockout mice line was generated. We observed a reduced litter size, a high infant mortality, frequent congenital phenotypic abnormalities in this mouse line, and moreover, an increased de novo germline mutation rate. These results suggest that oxidative DNA repair system plays an important role for the prevention of both somatic and germline mutations in mammals.

Mechanism of mitochondrial DNA replication in higher vertebrates

Whilst the majority of DNA is enclosed within the nucleus, mitochondria also contain a separate genome, the mitochondrial DNA (mtDNA). The significance of mtDNA can be recognized by the fact that mutations in mtDNA are associated with a variety of human disease. A series of recent studies proposed the presence of mtDNA replication intermediates which appear to be derived from two θ-type replication mechanisms in higher vertebrates. One is a unique replication mechanism: while the nascent H-strand (leading strand) is DNA, ribonucleotides are incorporated throughout the nascent L-strand and they are replaced by DNA after considerable delay. Replication intermediates of the other mechanism has properties of coupled leading- and lagging-strand DNA synthesis. These features indicate that the mammalian mtDNA replication system is more complex than previously considered, and thus it needs to be studied further. In this lecture the current model of mtDNA replication will be discussed.
Maintenance of the nucleotide pool in *C. elegans* under oxidative stress—Sanitization of oxidized nucleotides to maintain growth, development and survival in *C. elegans*

Reactive oxygen species (ROS) are continually generated during normal cellular metabolism. ROS oxidize nucleotide pool and generate various kinds of damages. The oxidized form of nucleotides (dNTPs) could be incorporated into DNA during DNA replication. To prevent mutation generation, many organisms possess enzymes to sanitize the oxidized dNTPs. In this study, to clarify the mechanism for sanitizing oxidized dNTPs and its roles in maintaining the genome stability in *C. elegans*, we focused on three proteins Ndx-1, Ndx-2 and Ndx-4 as players in sanitization of oxidized nucleotides. The substrate specificity of these enzymes, the phenotypes of RNAi-knockdown or mutant worms were investigated in *C. elegans*. We found that the sensitivity of these deficient worms to ROS largely increased and the triple mutants were highly sensitive to oxidative conditions in adulthood. These findings indicate that the Ndx enzymes play critical roles in defense against oxidative stress in *C. elegans*.

Histone H3 lysine 36 methyltransferase controls the programmed DNA-damage response

Diverse histone modifications are catalyzed and recognized by specific proteins, establishing unique modification patterns that act as chromatin signals. In particular, histone H3 di/trimethylation at lysine 36 (H3K36me2/3) is associated with actively transcribed regions, however, the control mechanisms and functions of H3K36me in higher eukaryotes are still enigmatic. We previously demonstrated that a H3K36 methyltransferase Wolf-Hirschhorn syndrome candidate 1 (WHSC1), also known as NSD2 or MMSET, functions for transcriptional regulation together with developmental transcription factors and that the Whsc1 deficiency is responsible for WHS. Here, we show that Whsc1 is required for normal B-cell development, which is coupled programmed DNA-damage response at the V(D)J regions.

We found that Whsc1 does not accumulate at exogenous DNA double-strand break sites (DSBs), but associates with DNA-damage response factors without exogenous DNA-damage. We propose that Whsc1 links transcription and DNA damage repair for maintenance of genome integrity.
Development of identification method based on cross-sectional analysis of transcriptome database for conserved peptide uORFs and its application to animal and plant genomes

Upstream open reading frames (uORFs) are often found in the 5'-untranslated regions of eukaryotic messenger RNAs. Some uORFs have been shown to encode functional peptides involved in the translational regulation of the downstream main ORFs. Comparative genomic approaches have been used in genomewide searches for uORFs encoding bioactive peptides, and by comparing uORF sequences between a few selected species or among a small group of species, conserved peptide uORFs (CPuORFs) have been identified in plants, mammals and insects. Detection of homology between such short regions largely depends on the selection of species for comparison. To maximize the chances of identifying CPuORFs with short conserved regions, we devised a novel algorithm for homology search among a large number of species and the automatic selection of uORFs conserved in a wide range of species. In this study, we developed the ESUCA method derived from our original method, BAIUCAS. ESUCA has several advantages over BAIUCAS. We identified over 300 novel homology groups of uORFs from plant and animal kingdoms by this application into 5 plant and 4 animal genomes.

Identification of Arabidopsis upstream open reading frames that control gene expression in a peptide sequence-dependent manner and analysis of their regulatory mechanisms

Upstream open reading frames (uORFs) are small ORFs present in the 5'-untranslated regions (5'-UTRs) of many eukaryotic mRNAs. The presence of uORF can negatively modulate translational efficiency of the downstream main ORF. Although the effects of most uORFs are independent of their encoded peptide sequences, some uORFs encode regulatory peptides that repress translation of the main ORF. For genome-wide identification of uORF-encoded regulatory peptides, exhaustive searches for uORFs with evolutionarily conserved amino acid sequences, referred to as conserved peptide uORFs (CPuORFs), have been conducted using bioinformatic analyses. To identify uORFs encoding regulatory peptides in Arabidopsis thaliana, we searched for CPuORFs showing a sequence-dependent regulatory effect by using transient expression analysis and in vitro translation analysis. From these screens, we identified twelve CPuORFs that repress main ORF expression in a peptide sequence-dependent manner. Furthermore, we investigated physiological roles of these sequence-dependent regulatory uORFs and found that post-transcriptional regulations mediated by uORF-encoded peptides play various physiological roles in plants.
Monitoring and gene regulation of the membrane protein biogenesis factors mediated by a translation arrest mechanism of an upstream ORF

The biogenesis of membrane proteins is an essential cellular function. *Bacillus subtilis* has two YidC homologs (SpoIIIJ and YidC2), which are members of conserved membrane protein biogenesis factors. While SpoIIIJ is expressed constitutively, YidC2 is induced when the activity of SpoIIIJ is compromised. The monitoring of the cellular YidC activity as well as gene regulation of YidC2 are both mediated by an upstream ORF *mifM* by the mechanism involving regulated translation elongation arrest of the nascent MifM polypeptide. I would like to introduce our recent studies on the molecular mechanism of the monitoring and gene regulation of YidCs by the uORF *mifM*.

Analysis of Epigenetic gene regulation of *DDI2* and *DDI3* caused by fluctuation of heterochromatin

We had analyzed “boundary”, control the extension of heterochromatin, which regulated the expression of genes at the close of heterochromatin region. In this study, we focused on *DDI2* and *DDI3* that regulated by fluctuation of heterochromatin under DNA damage. We constructed yeast strain which had ECFP, EYFP at the position of *DDI2*, *DDI3* ORF respectively. Then, we performed single cell analysis using MMS (methyl methansulfonate), which makes DNA damage, and traced expression level of *DDI2*, *DDI3* by detecting Fluorescence intensity. As a result, we found that the timing of expression was different even in genetically identical cells and these genes could not express by MMS in the *gcn5*Δ strain. Furthermore, our results suggested that the expression of *DDI2* and *DDI3* synchronized in the same cell, even though they exist on different chromosomes.

Decoding epigenetics related to distinct phases of long-term memory in Drosophila

Memory is consolidated to a stable form of memory, long-term memory (LTM), which process requires de novo gene expression mediated by epigenetic regulation. The formed LTM is either properly maintained or extinguished due to the environmental change. We show that different sets of epigenetic regulators were involved in distinct phases and LTM, formation, maintenance and extinction. We decoded epigenetics related to these phases, and determined their target genes. Abundant genetic resources in fly allowed us to examine whether the target genes are involved in individual phases of LTM. We found that the identified genes are required for either LTM formation, maintenance or extinction, supporting the view that those different phases of LTM are distinct epigenetic regulators and their target genes. This work provides central players of epigenetics regulating distinct phases of LTM, and also suggest a novel concept in which terminally differentiated neurons are still epigenetically processed to adjust the animals’ behavior to the changeable environment.
Paternal transmission of restraint-stress-induced epigenome change in Drosophila

Previously, we showed that Drosophila transcription factor-2 (dATF-2) is involved in heterochromatin formation and that phosphorylation of dATF-2 by various stresses, such as heat shock and osmotic stresses, induces heterochromatin disruption. We also revealed these stress-dependent epigenome changes mediated by dATF-2 can be inherited to next generations (Seong et al., 2011).

In mammal, restraint stress (RS) is an experimental model for psychological stress and is able to induce neuronal degeneration and some behavioral defects. We observed that Drosophila F1 progenies, derived from RS exposed fathers, showed significant disruption of heterochromatin state and that such effect was not observed in the dATF-2 and MEKK1 mutant background. Interestingly, RS exposure was able to induce expression of some drosophila cytokines and their target genes. Furthermore, we observed that RS exposure did not induced expression of target genes in the MEKK1 mutant background, while silencing of the target genes was abrogated in the dATF-2 mutant background. These data suggest that cytokine induction by RS might be required for dATF-2 pathway dependent epigenome change and its inheritance.

HP1a functions to suppress the evolutionarily fixed and telomeric transposons via piRNAs

The Piwi-interacting RNAs (piRNAs) are a class of small RNAs that protect the germline cells in animals from transposable elements (TEs). piRNAs function to suppress TEs post-transcriptionally in cytoplasm and silence the TEs transcriptionally in nucleus. Some chromatin-associated proteins are known to silence the TEs downstream of piRNAs. In the present study we show that HP1a is required uniquely for piRNA production and suppression of a specialized class of TEs. HP1a loss in Drosophila germline selectively causes derepression of telomeric TEs and some TEs in pericentric region, but not most of other transposons. Concomitantly, HP1a loss leads to severe reduction in telomeric transposon-mapping piRNAs and also loss of piRNAs mapping to some pericentric TEs, but only a feeble reduction in overall piRNA levels. Upon the loss of HP1a, the steady state levels of cluster transcripts and anti-sense transposons were not affected, and transport to the nuage component, Vasa, was not affected. We propose that HP1a actively represses the evolutionarily fixed and telomeric transposons in Drosophila germline via piRNA pathway. We will discuss potential mechanism underlying this phenomenon.

Molecular function of Tet1 in the epigenetic reprogramming through mouse germ cell development

Mouse germ cells are derived from epiblast somatic cells as primordial germ cells (PGCs) at peri-implantation stage. During PGC development, global epigenetic state is reprogrammed from somatic to germ cell type. Although these global changes, including genome-wide DNA demethylation, have been well-described, underlying mechanism and biological significance were unclear. We revealed that 5-methylcytosine (5mC)-specific deoxygenase, Tet1 plays an important role in PGC reprogramming. Although Tet1 is not important for the global demethylation, Tet1 is indispensable not only for the activation of meiotic genes, but also for the erasure of genomic imprinting. Furthermore, we found that Tet1 oxidizes 5mC of pericentromeric heterochromatin (PCH) in PGCs. Usually, Tet1 only localizes to open chromatin, and is excluded from PCH in somatic nuclear. PGC-specific localization to PCH of Tet1 may contribute to suppress transcription of major satellite repeat from PCH. In this talk, I’d like to discuss about the epigenetic reprogramming of PGC genome by focusing on the function of Tet1.
Reconstitution in vitro of the entire cycle of the mouse female germ line

The germ cell lineage ensures the creation of new individuals, thereby perpetuating the genomic and epigenetic information across the generations. During germ cell development, biologically significant events such as meiosis and gametogenesis are tightly controlled and disorder of the events causes infertility and developmental arrest of the next generation. Reconstitution of germ cell development in culture using pluripotent stem cells would provide a useful platform for understanding of molecular mechanisms underlying germ cell development. We recently established a culture system that induces functional mouse primordial germ cells as well as oocytes from pluripotent stem cells. There are, however, many issues to be overcome toward the robust generation of mature gametes or for application of the culture system to other species. In this meeting, I will discuss recent advances in the development of in vitro gametogenesis and introduce some experiments that are about to address a fundamental question of mammalian oogenesis.

Analysis of the mechanism that determines the direction of nuclear migration that separates mesoderm and endoderm fates

The mechanism that separates mesoderm and endoderm fates has been studied in various model animals, but its details are not understood well at the single-cell level. We have previously investigated this mechanism in tunicates and revealed that asymmetric localization of mRNA encoding Not and subsequent partitioning of the mRNA to the mesoderm precursor separates the fates. The localization of Not mRNA was coupled to the movement of the nucleus. We found that the destination of the nuclear migration, which is equivalent to the future mesoderm region, was initiated by re-localization of PI3K to the future mesoderm region via ooplasmic segregation. This polarity was maintained by PI3K signaling during later stages. Activity of PI3K, which remained localized to the future mesoderm region, determined the destination of nuclear migration. I am currently investigating the PI3K-dependent mechanism that instructs the nucleus toward the localized PI3K. Here, I will report the arrangement of microtubules during nuclear migration and its relation to PI3K signaling.

Molecular mechanisms of endoderm differentiation by Nodal signaling via nuclear movement

It has been well established that Nodal, a TGF- superfamily member, is an essential growth factor for mesoderm and endoderm (mesendoderm) induction before gastrulation in mouse and zebrafish embryos. Genetic analyses using zebrafish uncovered that two Nodal-related genes, Nodal-related 1 (ndr1) and Nodal-related 2 (ndr2), and EGF-CFC family gene teratocarcinoma-derived growth factor 1 (tdgf1), encoding a co-receptor of Nodal, are necessary and sufficient for mesendoderm induction. In addition, analyses using various zebrafish mutants and knockdown experiments have revealed that Sox32/Casanova is an essential transcription factor for endoderm fate determination downstream of Nodal signaling and the molecular pathways lead to endoderm fate. During the course of our analyses on endoderm specification, we found the marginal cells, which are the first fated to differentiate into endoderm. We will discuss the mechanisms of endoderm fate determination in these cells.
Regulation of MKL1 via actin cytoskeleton dynamics drives adipocyte differentiation

Cellular differentiation is regulated through activation and repression of defined transcription factors. A hallmark of differentiation is a pronounced change in cell shape, which is determined by dynamics of the actin cytoskeleton. Here we show that regulation of the transcriptional coactivator MKL1 (megakaryoblastic leukemia 1) by actin cytoskeleton dynamics drives adipocyte differentiation mediated by peroxisome proliferator-activated receptor γ (PPARγ), a master transcriptional regulator of adipogenesis. Induction of adipocyte differentiation results in disruption of actin stress fibers through down-regulation of RhoA-ROCK signaling. The consequent rapid increase in monomeric G-actin leads to the interaction of G-actin with MKL1, which prevents nuclear translocation of MKL1 and allows expression of PPARγ followed by adipogenic differentiation. Moreover, we found that MKL1 and PPARγ act in a mutually antagonistic manner in the adipocytic differentiation program. Our findings thus provide new mechanistic insight into the relation between the dynamics of cell shape and transcriptional regulation during cellular differentiation.

Mechanical control of gene expression via cytoskeleton

Recently, we have found that mechanical signals, such as stretch and shear stress, can regulate gene expression directly. This new type of regulation is fast and robust enough to change even cell fates. Nonetheless, precise molecular mechanisms underlying this are largely unknown. When cells are stretched by physical forces, cells are deformed. This physical deformation could affect molecular structures of protein components of the cytoskeletons, adhesion sites and nucleus/chromatin. We summarize our findings to review the physico-genetic loop of gene regulation in this workshop.
Strain-specific mutation underlying genetic background modifies hearing phenotypes in mice

Jackson shaker (Ush1g<sup>+/</sup>) exhibits abnormal behavior and congenital deafness owing to a homozygous mutation in the Usher syndrome type 1G gene (Ush1g). We found that C57BL/6J (B6J)-Ush1g<sup>+/−</sup> heterozygous mice exhibit early-onset progressive hearing loss (ePHL) with progressive stereocilia degeneration in the cochlear outer hair cells. ePHL did not develop in C3H/HeN mutant mice, suggesting that other genetic factors are required for ePHL development. Therefore, we performed classical genetic analyses and found that the occurrence of ePHL in Ush1g<sup>−/−</sup> mice was associated with a region on chromosome 10, which harbored the cadherin 23 gene (Cdh23). To confirm the effects of this mutation, we generated B6J-Ush1g<sup>+/−</sup> and Cdh23<sup>−/−;Ush1g−/−</sup> double-heterozygous mice using CRISPR/Cas9-mediated Cdh23<sup>−/−;Ush1g−/−</sup> knock-in mice. Cdh23<sup>−/−;Ush1g−/−</sup> mice harbored a one-base substitution (A for G), and the homozygous A allele resulted in a moderate hearing loss with aging. Analyses revealed complete recovery of ePHL and stereocilia degeneration in B6J-Ush1g<sup>−/−</sup> mice. These results suggest that epistasis between the Ush1g mutation and Cdh23 alleles leads to the development of ePHL in B6J-Ush1g<sup>−/−</sup> mice.

Triple-target CRISPR enabled almost perfect whole-body bi-allelic knockouts at first generation

The efficient production of biallelic KO mice can be facilitated by recently developed genome editing techniques, including the CRISPR/Cas nuclease system. However, two problems remain: (1) first-generation mice often contain a mosaic of wild-type and KO cells, and (2) the rate of complete biallelic mutant mice generated is relatively low (usually 76%–80% at best). In order to achieve almost complete biallelic knockout at first generation, we improved CRISPR/Cas system by developing a new method to design gRNAs with high efficiency and low off-targets, and utilizing three gRNAs within a target gene to obtain maximum knockout efficiency. As a result, the triple-target CRISPR method elicited almost perfect (98%–100%) whole-body KO of the Tyrosinase (Tyr) gene, which is functionally evaluated by animal coat color. This KO efficiency was confirmed using three independent sets of gRNAs. These results demonstrate that the triple-target CRISPR method enabled highly efficient gene KO phenotype screening with less efforts and fewer animals.
Visualization of ATP dynamics in vivo

Adenosine 5’-triphosphate (ATP) is the major energy currency of cells and is involved in many cellular processes, such as cell motility and development. However, there is no method for real-time monitoring of ATP levels inside individual living cells. In 2009, two new methods were reported, one is a series of Forster resonance energy transfer (FRET)-based indicators for ATP, ATeam and other is a engineered fluorescent sensor of ATP and ADP, Perceval. I generated the ATP visualization mouse using modified ATeam. In my talk, I will show and discuss the dynamics of ATP metabolism in vivo.

YfdR protein encoded in a cryptic prophage of the E. coli genome binds to DnaA protein, the initiator of chromosome replication

The gene cluster of yfdQRST is present in the cryptic prophage CPS-53 of the E. coli K12 genome, and has 97% identity in nucleotide sequence to the orf28-32 of the bacteriophage SfV of Shigella flexneri. However, the precise roles for these genes remain to be explored. We have found that the chromosomal replication initiator DnaA protein directly binds to a prophage gene product YfdR protein. Mutant analyzes suggested that YfdR as well as DnaB helicase and DiaA (a stimulator of DnaA assembly) binds to a specific site within DnaA domain I. Consistently, YfdR inhibited binding of DnaA with DnaB or with DiaA. When the yfdQRST genes are oversupplied in E. coli cells, synthetic lethality with certain dnaA-temperature sensitive mutant alleles occurred.

Nasal immunization with plasmid DNA encoding P6 protein elicits nontypeable Haemophilus influenzae-specific long-term mucosal immune responses in the nasopharynx

Nasal vaccination is an effective therapeutic regimen for preventing upper respiratory infection, while DNA vaccines represent a new approach for controlling infectious diseases. The efficacy of nasally administered DNA vaccine was examined. A DNA plasmid encoding the P6 protein of nontypeable Haemophilus influenzae (NTHi) was constructed. Mice were immunized intranasally with the DNA plasmid, and P6-specific immune responses were examined using purified P6 protein. Nasal CD4+ T cells were purified and incubated in the presence of P6, and the expression of cytokine mRNA was examined. In addition, NTHi challenges were performed and the bacterial clearance was examined. P6-specific nasal wash IgA and serum IgG were elevated following immunization with the DNA vaccine. The specific IgA-producing cells increased in the nasal passages. In addition to Th1 and Th2 cytokine expression, IL-17 was detected in P6-specific T cells. Moreover, DNA vaccination enhanced bacterial clearance. These findings suggest that nasal vaccination with P6 DNA vaccine might be a new effective regimen for the induction of specific protective immunity in the upper respiratory tract.
The chromosomal replication of virus, fungi and parasite

I have been studied for Polyoma virus (Py) and budding yeast. The Py is cause tumors in mice. Large T antigen (LT) coded in Py is a powerful oncogene and is a helicase necessary to Py genome replication.

The Py genome proliferation is also activated by an external stimulus. And transcription factors such as Ets AP1, Runx / CBF bind to the early region of the replication origin of Py genome origin and regulate the Py genome replication(1-3).

I have shown that budding yeast chromosomal replication is regulated by the transcription factors(1, 4). On the other hand, surprisingly, the presence of gene amplification has been suggested in the replication of certain parasite genome. In this presentation, I’d like to discuss chromosomal replication apparatus and the disease of virus, fungi and parasites(5).


An interaction between intestinal IgA and intestinal microbiota

Immunoglobulin A (IgA) is the main antibody isotype secreted into the intestinal lumen. It plays a critical role in the defense against pathogens and in the maintenance of intestinal homeostasis. However, the molecular mechanisms of how secreted IgA regulates intestinal microbiota are not completely understood. We generated IgA-producing hybridomas derived from the intestinal IgA-secreting cells of unimmunized wild-type mice. We also identified their target bacterial epitopes. Unexpectedly, more than 90% of monoclonal IgAs recognized an epitope that represented four amino acids (EEHI) expressed in a bacterial enzyme, serine hydroxymethyltransferase (SHMT). The intestinal IgA reacting the EEHI sequence seemed to be preferentially selected in vivo. Among those IgAs, we selected a high-affinity polyreactive W27 IgA. W27 selectively binds to colitogenic/pathogenic bacterial species such as Escherichia coli and Pseudomonas fulva with the strict recognition of the EEHI motif in SHMT, while ignoring the beneficial ones (Bifidobacterium bifidum, Blautia coccoides and Lactobacillus casei). Thus we show a plausible molecular mechanism how intestinal IgA regulates gut microbiota.

Oral microorganisms and disease prevention

Chronic diseases in the mouth or in the epiglottis sometimes causes other diseases in some other parts of the body that are not connected to the mouth. These diseases are called focus diseases and at present various diseases such as IgA nephrosis, rheumatism, palmoplantar pustulosis and so on have been reported. And it is also said metabolic syndrome including diabetes is connected to chronic inflammation in the mouth. We can avoid suffering from these focus diseases by preventing serious ailments like periodontal diseases and tooth decay with proper treatment. We are convinced of the possibility of effectively eliminating these focus diseases by using the following three procedures.

• Using the power of the sterilized water in the dental treatment
• Using the proteolytic type bacterial elimination water for home care
• Using the pulse width-variable and high peak-pulse type Nd-YAG laser
Profiles of DNA replication fork progression in *Escherichia coli*

As genome presents many obstacles to replication fork progression, DNA replication is frequently impaired in cells. The impairment provokes replication stress, the primary cause of genomic instability. Thus, understanding genomic stability requires a genome-wide approach to studying DNA replication. However, fine dynamics of replication forks on genome has not been well elucidated in *Escherichia coli*, the leading and the simplest mode of studying DNA replication. To overcome this difficulty, we created a novel thymidine-requiring *E. coli* strain, eCOMB, which can incorporate the thymidine analog BrdU rapidly and efficiently enough to permit pulse labeling of nascent DNA with BrdU in vivo. Analysis of the BrdU-labeled nascent DNA showed that the speed of individual forks is relatively uniform on the genome obstructive to their progression, and that *E. coli* cells significantly reduce the speed of unperturbed replication forks during the bacterial DNA damage response. Furthermore, a genome-wide replication profile in the exponentially growing cells showed that replication forks are paused in two 200-kb chromosomal zones flanking the replication origin.

Genome expression regulation in *Escherichia coli* K-12

Bacteria are directly exposed to various stressful environments and hence have sophisticated stress response systems for survival. For the adaptation to various external conditions, bacteria change the expression pattern of its genome mainly at the step of transcription. The selective transcription takes place by controlling the promoter recognition properties of RNA polymerase. Comprehensive analyses of *E. coli* genome show that transcription factors classify two groups of a local and global regulator, forming the hierarchical multi-regulatory network. This regulatory network seems to play a role for *E. coli* survival to colonize in a large intestine within host animals. Also we identified non-annotated promoters regulated by a transcription factor. Here we will discuss the transcriptional regulatory network of *E. coli* with novel identified genes.

An alternative pathway of chromosome replication in *Escherichia coli*

E.coli cells can replicate its genome in the absence of DnaA-oriC under some genetic or environmental backgrounds. Most prominently, replication in the absence of RNaseH can support cell growth in the absence of DnaA-oriC. This alternative mode of replication requires transcription, RecA and replication factor, PriA. We show that PriA binds to ter region when this system is operative, and ter becomes essential for growth in the absence of DnaA-oriC. Further mapping led to identification of two loci that are essential. One is dif and it is probably required for proper decatenation of replicated chromosomes. The other, termed oriT1, may be required for efficient initiation of replication of the genome. Analyses of cis- and trans-acting factors required for this mode of *E.coli* replication may provide novel insight into the modes of replication initiation in higher eukaryotes including human genome. We would like to thank Tsutomu Katayama, Kazutoshi Kasho, Yoshito Abe, Nobuaki Kono, Taku Ohshima, Junichi Kato, Takashi Hishida and Masami Hidaka for various purified proteins, strains and analyses of the genome-wide data.

Maintenance for timely initiation of DNA replication via dynamic regulation of the complexes of the nucleoid protein IHF and specific DNA elements

In *Escherichia coli*, chromosome DNA is folded, constructing dense ‘nucleoid’. A nucleoid protein IHF plays specific roles in nucleoid folding and regulating various cellular events including DNA replication, transcription and recombination. As to DNA replication, IHF binds to non-coding DNA elements such as *DARS2* and *datA* for positively and negatively regulating DnaA initiator protein (Kasho et al., 2013 & 2014), as well as the replication origin *oriC*. In this study, we analyzed IHF binding dynamics to specific sites in these elements. Based on these, we will discuss the timely regulation of IHF binding dynamics.
The role of H-NS in the diversification of the sequences in the horizontal transferred DNAs

Bacteria acquired foreign genes, which made bacteria adapt to highly diversified environments on the earth. We performed ChIP-seq analysis for E. coli silencer, H-NS, to determine their binding regions in chromosomes of three E. coli strains and compared the DNA sequences in the identified H-NS binding regions. The analysis showed that the DNA sequences in the conserved H-NS binding regions among these strains were more diversified than those of H-NS unbound regions. In addition, the mutations of H-NS binding regions diversified transcriptional intensities among E. coli strains. These results indicated that H-NS contributes to diversify DNA sequences and transcriptional activities in H-NS binding regions during the E. coli evolution. This suggests that the foreign genes have been incorporated in the host transcriptional network during the E. coli evolution under the transcriptional silencing with H-NS.

Education for Synthetic Biology at universities

In synthetic biology, researchers need to combine (1) theoretical basis in information science and control engineering and (2) biological knowledge and experiment techniques. For education of such combination, iGEM is an ideal occasion. Because iGEM teams have only less than one year for the activity, education in each university before stating iGEM activity is quite important. However, such interdisciplinary education is rarely provided in Japanese universities. Students are thus required to find suitable combination of classes. From my viewpoint established through 10-years iGEM activity and experience in interdisciplinary department, I will introduce a model example of combination.

Degradation of uric acid by E.coli

In Japan, environmental pollution caused by excrement of birds is a problem that should be solved. For example, dieback of trees and spoiling the cityscape are major problems. Birds’ dropping consists mainly of uric acid and that is really insoluble and uric acid can be degraded to soluble material, urea. So, our goal is to catalyze uric acid to urea and make it possible to wash away dropping by rainwater. We’d like to lead our project to the solution of the pollution. Considering purine metabolism pathway, three enzymes, urate oxidase, allantoinase, allantoicase, must be synthesized to degrade uric acid to urea.
WS10 SAIITO, Kensuke1, YAMAZAKI, Ken-ichi1 (1Crs. Mol. Biol., Grad. Sch. Env. Sci., Univ. of Hokkaido)

The Gel-free BioBrick isolation (GFBI)

Both edges of BioBricks should be processed appropriately for gene assembly through BioBrick-fusion. DNA sequence-specific restriction endonucleases are frequently used for that purpose. Gel-electrophoresis is commonly used for isolation of processed BioBricks. Beginner scientists frequently make mistake even on this isolation step, and the step is also time consuming. Here we introduce new method for BioBrick isolation free from gel-electrophoresis by employing avidin-conjugated magnetic beads and biotinylated DNA primer. We could isolate BioBrick with high yield by simple manipulation in a minute. Every scientist can prepare BioBricks suitable for gene assembly without any mistake by the gel-free BioBrick isolation (GFBI).

WS10 MICHIMORI, Yuta1, MATSUMOTO, Sora1, YAMADA, Syuhei1, KOIDE, Eri1 (1UnderGrad. Sch. Ind. Chem., Facilt. Eng., Kyoto Univ.)

Detection of Norovirus by E.coli Expressing Surface-displayed Recombinant Protein with Specific Adhesion to Norovirus

Even though Norovirus (NoV) remains the leading cause of food poisoning in the developed countries, the world has yet to discover an anti-NoV vaccine, or its curative medicine. As an alternative approach to this global issue, iGEMKyoto is developing an intestinal bioelimination system that physically removes NoV particles from the body. In detail we are creating recombinant E. coli expressing surface-displayed proteins that each specifically bind to NoV and cellulose. Additionally, we are also considering the application of the said E. coli to NoV prevention and detection. Through the summer 2015 and spring 2016 experimentation, we have already constructed the said E. coli, and confirmed its affinity to NoV particles from Scanning Electron Microscopy. This summer, we plan on further examining the characteristics of this device, as well as testing it for new applications.

WS10 OTSUBO, Takuho1, TAKEKAWA, Kyosei1, KANEDA, Yuhei1, YAMAMOTO, Shinsei1, OGURA, Atsushi1, OTAKI, Yoshiharu1, SAITO, Naoki1, HARAGUCHI, Duki1, MINEI, Ryuhei1 (1Bio Sci. Biol. Sci. Dept., Nagahama Inst. Bio-Sci. Tech.)

Increased production of fragrance for new food preservation method FLAVORATOR® by odor E. coli.

Food problems are a serious matter in the world. Among them, food preservation is the one. Ideal food preservation is keeping food without causing quality change cost-effectively for longer time. Many preservation methods have been used so far. Here, we created a new one to solve it. It is “Flavorator”!! Recombinant E. coli produces antimicrobial and insecticidal volatile gaseous substances that suppress unwanted bacterial growth in “Flavorator” and prevent insects from entering “Flavorator”. Our choice was geraniol and farnesol. The reason for our choice is that they are terpenes and can be produced by E. coli easily after engineering its metabolic pathways. And they have high antimicrobial activity. Geraniol and farnesol are the major constituents of rose fragrance. These fragrances not only harmlessly emit fragrant odor to humans but also kill/or suppress microbes as well as insects like fruit flies. We designed our strategy following three sequential steps in order to realize “Flavorator”.

Step 1: Increase in the amount of terpene’s precursors.
Step 2: Production of geraniol and/or farnesol.
Step 3: Efficient export of geraniol and/or farnesol to the media.

Following these three steps, we are sure that we will achieve realization of concept of “Flavorator” that can avoid food decay and solve one of the food problems in the future.


Creating a genetic circuit functional in E.coli which allows the gene expressed to change after each cell division

We aim to create a genetic circuit functional in E.coli which allows the gene expressed to change after each cell division. Specifically, the E. coli will be expressing GFP, RFP, and CFP after subsequent cell divisions and will loop back to expressing GFP again. This will be achieved by the use of the Pnrd promoter to sense to cell division, sigma factor/anti-sigma pairs control transcription, and a toehold switch to create an AND gate required in the circuit. Our project would be able to exhibit, in E. coli, that despite carrying the same genetic information, different phenotypes can be expressed across generations. Much like the phenomenon seen in epigenetics. Moreover, this technology could potentially be applied in field to carry out certain tasks automatically without the need of external input eg. to make engineered cells automatically carry programmed cell death after a certain number of divisions.
Circularization of a protein is one of the ways to enhance its stability against various temperatures and pH levels. We challenged ourselves to circularize proteins using self-assembling peptides and linkers. Self-assembling peptide (SAP) is an amphiphilic peptide which self-assembles under physicochemical conditions. We made SAPs fused to link ends of the protein. We also inserted cysteine residues in the linker which is essential for the circularization so that they form a disulfide bond. This linker is expected to be applicable to most proteins because it automatically adjusts its length. These two elements - the self-assembling peptide and the linker - enable the protein to be circularized.

Relationship between the initiation reaction of DNA replication and chromatin regulator in eukaryotes

DNA, the carrier of genetic information in organisms, is stored as chromosomes, which is consists from many chromatins, in nuclei in eukaryotes. It is widely known that there are regulatory mechanisms that cooperate chromatin regulating factors and DNA-mediated reactions, such as transcription and DNA repair. This can let us expect that the existence of cooperative mechanism(s) between chromatin regulators and DNA replication, which is a essential process for cell proliferation. However, this is largely unknown, although some study have shown that the contribution of chromatin regulators to replication licensing and elongation process of DNA replication. Moreover, the contribution of chromatin regulators onto the initiation reaction of DNA replication is totally unknown. Here we would like to show some results indicating a chromatin regulator has a role in the initiation of DNA replication. Our results reveal novel level of regulation of DNA replication.