Department of Basic Medical Sciences

Division of Molecular Cell Signaling
分子細胞情報分野

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Protein phosphorylation and dephosphorylation are among the most important intracellular signaling mechanisms, and are mediated respectively by protein kinases and protein phosphatases. We study various aspects of cellular signal transduction with a particular emphasis on the role and regulation of protein phosphorylation and dephosphorylation in cellular stress responses, using both mammalian and yeast cells.

1. Dynamic control of yeast MAP kinase network by induced association and dissociation between the Ste50 scaffold and the Opy2 membrane anchor

Katsuyoshi Yamamoto[1], Kazuo Tatebayashi, Keiichiro Tanaka and Haruo Saito: [1]Frontier Research Initiative, IMSUT

In eukaryotic cells, various extracellular stimuli generate intracellular signals that converge on a limited number of conserved protein kinase cascades, commonly referred to as mitogen-activated protein kinase (MAPK) pathways. Each MAPK is activated through a cascade of three successively activating protein kinases: MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK.

The budding yeast *Saccharomyces cerevisiae* survive widely fluctuating osmotic conditions in their natural habitat such as ripening fruits. To cope with an increased external osmolarity, yeast temporarily arrest cell cycle progression, readjust transcription and translation patterns, and synthesize and retain the compatible osmolyte glycerol. These adaptive responses are governed by the High Osmolarity Glycerol (HOG) signaling pathway, whose core is the Hog1 MAPK module.

The upstream part of the HOG pathway comprises the functionally redundant, but mechanistically distinct, SLN1 and SHO1 branches. A signal emanating from either branch converges on a common MAPKK, Pbs2, which is the specific activator of Hog1. The SLN1 branch activates the redundant Ssk2 and Ssk22 MAPKKKs, which then activate Pbs2. The SHO1 branch activates the Ste11 MAPKKK, which then activates Pbs2. Thus, a mutant that lacks both the *SSK2* and *SSK22* genes (an *ssk2/22*Δ mutant) is totally dependent on the SHO1 branch for activation of the Hog1 MAPK.

A response in the SHO1 branch is initiated by the putative osmosensors Msb2 and Hkr1, which are highly glycosylated single-pass transmembrane (TM) proteins. This response leads to activation of the PAK-like kinase Ste20 by inducing its association with the membrane-bound small G-protein Cdc42. Activated Ste20 then phosphorylates and activates Ste11, which in turn phosphorylates and activates Pbs2 that is associated with the Sho1 membrane anchor. Be-

cause both the Cdc42-Ste20 and the Sho1-Pbs2 complexes are on the membrane, Ste11 must also be localized to the membrane for efficient activator/substrate interactions to take place. Membrane localization of Ste11 is mediated by the Ste50 scaffold protein that forms a stable complex with Ste11, primarily via association of Ste50 with the membrane anchor protein Opy2.

This year, we showed that Opy2 has three Ste 50 binding sites (CR-A, -B, and -D) with different affinities, regulation, and functions. The affinity of CR-B to Ste50 increases in the presence of glucose as it is phosphorylated by the Casein Kinase I isoforms Yck1/Yck2. Furthermore, Ste 50 bound to CR-B can activate the HOG MAPK pathway, but cannot efficiently activate the FG/IG MAPK pathway. In contrast, CR-A, which allows both the HOG and the FG/IG responses, is the predominant binding site of Ste50 in a glucose-poor environment. CR-D contributed only marginally to Opy2-Ste50 interaction under our experimental conditions.

The interaction between Ste50 and Opy2 is also modulated by Ste50 phosphorylation. We found that Ste50 is phosphorylated by any of three MAP kinases (Hog1, Fus3, and Kss1), and phosphorylated Ste50 cannot bind any of the Opy2 sites (CR-A, -B, or -D). For the Hog1 MAPK pathway, Ste50 phosphorylation shortens the duration of Hog1 activation following osmostress stimulation, reduces the baseline responses in the absence of extrinsic stimuli, and inhibits the osmostress-induced Hog1 activation when the mating pathway has been already activated. For the Fus3/Kss1 MAPK pathway, Ste50 phosphorylation also reduces baseline responses in the absence of extrinsic stimuli.

Thus, modulation of Opy2-Ste50 interaction by phosphorylation of Opy2 and Ste50 dynamically regulates multiple MAPK pathways by integrating external nutritional signals, internal feedback signals, and signals from parallel MAPK pathways.

2. Oncogenic Ras abrogates MEK SUMOylation that suppresses the ERK pathway and cell transformation

Yuji Kubota, Pauline O'Grady, Haruo Saito, and Mutsuhiro Takekawa

In mammalian cells, the ERK-MAPK pathway mediates mitogenic signalling and is essential for the control of cell fate, differentiation, and proliferation. ERK signalling is initiated by activation of cell surface receptor tyrosine kinases, which then induce the small G protein Ras to exchange GDP for GTP. The Raf MAPKKK family is recruited to the plasma membrane and is activated by GTP-bound Ras. Activated Raf phosphorylates and activates MEK1 and MEK2, which then activate ERK1 and ERK2. Activated ERK translocates to the nucleus where it phosphorylates transcription factors to induce the expression of growth-promoting genes. Genetic alterations resulting in constitutive activation of ERK signalling are common in cancer cells. In particular, Ras proteins are activated by mutations in approximately 30% of all human cancers.

Small ubiquitin-like modifiers (SUMOs), 92-97 amino acid polypeptides, are important modulators of cellular functions. Four vertebrate SUMO isoforms, SUMO1-4, are known. The C-terminal glycine in processed SUMO covalently attaches to an internal lysine residue in substrate proteins via an isopeptide bond. Proper sumoylation involves an E1-activating enzyme consisting of an SAE1/SAE2 heterodimer, the E2-conjugating enzyme Ubc9, and diverse E3 ligases, which contribute to substrate selectivity. SUMO is removed from target proteins by cysteine proteases (SENPs), making sumoylation a reversible and dynamic process.

This year, we demonstrated that SUMO1-modification of MEK negatively regulates the ERK pathway and indicated its importance in carcinogenesis. MEK sumoylation strongly attenuates MEK activity towards ERK by disrupting the specific docking interaction between MEK and ERK, thereby inhibiting the ERK pathway. We found that MEK is highly sumoylated at the plasma membrane, where activated MEK is predominantly localized. This may explain why sumoylation of MEK so effectively downregulates ERK activity, even though the overall sumoylation of cytoplasmic MEK appears to be relatively low. The MEK mutants that are resistant to sumoylation, MEK1(K104R) and MEK2(K 108R), were more potent than their wild-type counterparts in activating ERK in vivo. Cells expressing the MEK1(K104R) mutant also exhibited enhanced differentiation, proliferation, and cell transformation properties, reflecting the higher ERK activity in those cells. Therefore, inhibition of MEK activity by sumoylation, in concert with protein phosphatase-mediated inhibition, may control the magnitude and duration of ERK activity.

Another important finding is that MEK sumoylation is altered under pathological conditions. In fact, oncogenic Ras abrogates MEK sumoylation. Thus, MEK sumoylation is totally absent in human cancer cell lines harbouring various oncogenic Ras mutations, and inhibition of Ras activity in those cells restores MEK sumoylation.

We also elucidated the mechanism by which

oncogenic Ras inhibits MEK sumoylation. We identified MEKK1 as a MEK-specific SUMO-E3 ligase, and found that oncogenic Ras greatly enhanced Ubc9-MEKK1 association. Because continuous cycles of recruitment and dissociation of an E2 and an E3 are a prerequisite for efficient conjugation of SUMO to substrates, our data imply that oncogenic Ras inhibits MEK sumoylation by inhibiting release of Ubc9 (E2) from MEKK1 (E3).

Based on these findings, we proposed that oncogenic Ras proteins promote ERK pathway activation by two distinct mechanisms. First, oncogenic Ras directly binds to and activates the Raf family MAPKKKs to initiate the ERK cascade. Second, oncogenic Ras abrogates MEK sumoylation and releases MEK from the sumoylation-mediated inhibition of the docking interaction with ERK, thereby facilitating efficient phosphorylation and activation of ERK. These two mechanisms may synergistically hyper-activate the ERK pathway, and eventually induce cell transformation and carcinogenesis.

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Department of Basic Medical Sciences

Division of Neuronal Network
神経ネットワーク分野

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Our major research interest is the molecular mechanisms of higher brain functions in mammals such as emotion, and learning and memory. We are especially focusing on the roles of functional molecules localized in synapses, for instance, neurotransmitter receptors, signal transduction molecules and adhesion molecules, in neuronal information processing. We are examining receptor functions, synaptic transmission and plasticity, and their roles in the whole animal with electrophysiological, biochemical, molecular genetic and behavioral approaches.

1. Plexin-A2 regulates spatial memory and pattern separation through structural modification of mossy fiber projection in the CA3 region of the mouse hippocampus

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The hippocampus has been implicated in certain types of memory, including spatial memory. It has been known that the distribution of mossy fibers, axons of dentate gyrus (DG) granule cells, is modified dynamically by spatial learning in living animals. However, the precise mechanism of the regulation of mossy fiber distribution during memory formation is not well understood. We have previously reported that plexin-A2 (PLA2), one of the type A plexins that show repulsive activities to the class 6 semaphorins, regulates the distribution of mossy fiber terminals in the CA3 region and that the mutant mice lacking PLA2 (PLA2$^{-/-}$ mice) exhibit a shift of mossy fibers from the suprapyramidal to the infra- and intrapyramidal regions. In order to test whether the difference in the distribution of mossy fiber terminals affects abilities of learning and memory, we have performed extensive behavioral analyses of PLA2$^{-/-}$ mice. We found that sensorimotor functions and emotional behaviors of PLA2$^{-/-}$ mice were normal, although motor learning was markedly impaired presumably through aberrant distribution of cerebellar granule cells, and that contextual and auditory fear conditioning, which is at least partially dependent on the hippocampus, was also intact. In contrast, PLA2$^{-/-}$ mice exhibited enhanced hippocampus-dependent spatial reference memory and spatial pattern separation, which is the ability to discriminate fine differences in external environments, tested by the 8-arm radial maze task. These results suggest that the projection of mossy fibers regulated by PLA2 may be a specific determinant of the ability of spatial reference memory and pattern separation.

2. Tyrosine phosphorylation of the GluN2B subunit of NMDA receptors regulates anxiety-like behavior and CRF expression in the amygdala

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Anxiety disorders are a highly prevalent and disabling class of psychiatric disorders. There is growing evidence implicating the glutamate system in the pathophysiology and treatment of anxiety disorders, though the molecular mechanism by which the glutamate system regulates anxiety-like behavior remains unclear. In this study, we provide evidence suggesting that tyrosine phosphorylation of the NMDA receptor, an ionotropic glutamate receptor, contributes to anxiety-like behavior. The GluN2B subunit of the NMDA receptor is tyrosine-phosphorylated: Tyr-1472 is the major phosphorylation site. Homozygous knock-in mice that express a Tyr-1472 -Phe mutant of GluN2B, which prevents phosphorylation of this site, show enhanced anxiety-like behavior in the elevated plus-maze test. Expression of corticotropin-releasing factor (CRF), which is important for the regulation of anxiety-like behavior, is increased in the amygdala of the knock-in mice. Furthermore, injection of CRF-receptor antagonists attenuates the enhanced anxiety-like behavior of the knock-in mice. We also show that elevated plus-maze exposure simultaneously induces dephosphorylation of Tyr-1472 and increased CRF expression. These data suggest that Tyr-1472 phosphorylation on GluN2B is important for anxiety-like behavior by negative regulation of CRF expression in the amygdala.

3. Age-dependent regulation of depression-like behavior by interleukin-1 through modification of adrenergic signaling

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Interleukin-1 (IL-1) plays a crucial role in stress responses and its mRNA is induced in the brain by stress load; however, the precise role of IL-1 in higher brain functions and their abnormalities is largely unknown. Here, we report that IL-1 receptor antagonist (IL-1Ra) knockout (KO) mice, which lack IL-1Ra molecules that antagonize the IL-1 receptor, displayed anti-depression-like phenotypes in the tail-suspension test (TST) and forced-swim test (FST) only at a young stage (8 weeks), whereas the phenotypes disappeared at later stages (20 and 32 weeks). These anti-depression-like phenotypes were reversed by the administration of adrenergic receptor (AR) antagonists against the $AR\alpha_1$, $AR\alpha_2$, and ARβ subtypes. Although the contents of 5-hydroxytryptamine, norepinephrine, and dopamine, which are known to be associated with major symptoms of psychiatric disorders, were not significantly different in the hippocampus or cerebral cortex between IL-1Ra KO and their wild-type littermate mice, the mRNA expression level of the $AR\alpha_{1A}$ subtype was significantly changed in the cerebral cortex. Interestingly, the change in the expression of the $AR\alpha_{1A}$ subtype was correlated with an age-dependent alteration in the TST and FST in IL-1 Ra KO mice. These results suggest that sustained activation of the IL-1 signaling induced by gene manipulation in mutant mice affects the expression of the $AR\alpha_{1A}$ subtype and that modification of the adrenergic signaling by the IL-1 system may ultimately cause significant psychiatric abnormalities such as depression and this mutant mouse could be regarded as a model animal of depression that specifically appears in children and adolescents.

4. The mechanisms of the strong inhibitory modulation of long-term potentiation in the rat dentate gyrus

Fumiko Arima-Yoshida, Ayako M. Watabe and Toshiya Manabe

The hippocampus is essential for the formation of certain types of memory, and synaptic plasticity such as long-term potentiation (LTP) is widely accepted as a cellular basis of hippocampus-dependent memory. Although LTP in both perforant path-dentate gyrus (DG) granule cell and CA3-CA1 pyramidal cell synapses is similarly dependent on activation of postsynaptic N-methyl-D-aspartate (NMDA) receptors, several reports suggest that modulation of LTP by γ-aminobutyric acid (GABA) receptor-mediated inhibitory inputs is stronger in perforant path-DG granule cell synapses. However, little is known about how different the mecha-

nism and physiological relevance of the GABAergic modulation of LTP induction among different brain regions are. We confirmed that the action of $GABA_A$-receptor antagonists on LTP was more prominent in the DG, and explored the mechanism introducing such difference by examining two types of $GABA_A$ receptor-mediated inhibition, synaptic and tonic inhibition. As synaptic inhibition, we compared inhibitory versus excitatory monosynaptic responses and their summation during an LTP-inducing stimulus, and found that the balance of the summated postsynaptic currents was biased toward inhibition in the DG. As tonic inhibition, or sustained activation of extrasynaptic $GABA_A$ receptors by ambient GABA, we measured the change in holding currents of the postsynaptic cells induced by $GABA_A$-receptor antagonists, and found that the tonic inhibition was significantly stronger in the DG. Furthermore, we found that tonic inhibition was associated with LTP modulation. Our results suggest that both the larger tonic inhibition and the larger inhibitory/excitatory summation balance during conditioning are involved in the stronger inhibitory modulation of LTP in the DG.

5. Functional coupling of the metabotropic glutamate receptor 5, IP_3 receptor and L-type calcium channel: A role in regulation of calcium dynamics and synaptic plasticity.

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Activity-dependent regulation of calcium dynamics in neuronal cells can play significant roles in the modulation of many cellular processes such as intracellular signaling, neuronal activity, and synaptic plasticity. Among many calcium influx pathways into neurons, voltage-dependent calcium channels (VDCCs) are the major source of calcium influx, but their modulation by synaptic activity has still been under debate. While metabotropic glutamate receptors (mGluRs) are supposed to modulate L-type VDCCs (L-VDCCs), their reported effects include both facilitation and suppression, probably reflecting the uncertainty of both the molecular targets of the agonists and the source of the recorded calcium signals in those previous reports. In this study, using subtype-specific knockout mice, we have clearly shown the mGluR 5-induced facilitation of the depolarization-evoked calcium currents. This facilitation was not accompanied by the change in the single-channel properties of VDCC itself; instead, it was caused by the activation of calcium-induced calcium release that is triggered by VDCC opening. This effect was blocked by the inhibitors of both L-VDCCs and IP_3 receptors (IP_3Rs), suggesting the specific functional coupling between the mGluR5, IP_3R and L-VDCC. Furthermore, we have shown the mGluR5-mediated enhancement of VDCC-dependent long-term potentiation of excitatory synaptic transmission. Our data identify a novel mechanism of the interaction between the mGluR and calcium signaling, and suggest a possible contribution of mGluR5 in synaptic plasticity.

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Department of Basic Medical Sciences

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遺伝子動態分野

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RNA no longer stands behind DNA or protein but stands in front of DNA and protein. Recent achievements and discovery in biological science clearly emphasize the importance of RNA in life; the discovery of RNA interference, molecular mimicry between protein and RNA, and ribosome structure at atomic resolution. Moreover, the completed human genome project revealed, to our great surprise, the existence of a large amount of protein-noncoding RNAs (ncRNAs). These ncRNAs can be classified into two types: one, like antisense and microRNA, those function with the sequence complementarity to the target mRNA or DNA, while the other, like aptamer, those function independent of the sequence complementarity. In our laboratory, we aim to: 1) uncover the natural aptamers encoded in human genome; and 2) create artificial aptamers to target proteins of therapeutic interest. By studying these natural and artificial RNA aptamers, we hope to clarify superior potential of RNA, which would be highly beneficial to the development of RNA medicine and the comprehensive understanding of human genome RNA function. In addition to these RNA oriented study, two lines of translation orientated studies are in progress: 1) the molecular mechanism of translation termination and the molecular basis of mimicry between translation factors and tRNA; and 2) the 'prion' nature associated with yeast translation factor Sup35.

1. RNA Aptamer against Interleukin-17 and its Therapeutic Potential

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An RNA aptamer is a biochemical or therapeutic agent that targets a given molecule that is selected by a process known as SELEX (systematic evolution of ligands by exponential enrichment) from a complex library of random RNA sequences of typically 10^{14} different molecules. The concept is based on the ability of short (20-80 mer) sequences to fold, in the presence of a target, into unique three-dimensional structures that bind the target with high affinity and specificity. Therefore, aptamer can be thought of as nucleic acid analog to antibody, and its therapeutic applications are rapidly developing worldwide.

Interleukin-17 A (IL-17 A) is a pro-inflammatory cytokine produced primarily by a subset of CD4+ T cells, called Th17 cells, that is involved in host defense, inflammation and autoimmune disorders. This study was undertaken to investigate the effect of an aptamer against human IL-17A on IL-17A-induced signal transduction in vitro and its anti-autoimmune efficacy in vivo in inflammation mouse models. By screening of a large library of nuclease-resistant RNA oligonucleotides, we selected an RNA aptamer, Apt21-2, that binds human and mouse IL-17s and blocks the interaction between IL-17A and its receptor. The inhibition of IL-17A -mediated phosphorylation and production of marker proteins was analyzed in human and mouse cells. Mice with GPI-induced rheumatoid arthritis and MOG-induced experimental autoimmune encephalomyelitis were used to assess efficacy. Apt21-2 prevented efficient phosphorylation of IL-17A signaling factors, IκB and JNK, and inhibited the production of interleukin -6 in human and mouse cells. A PEGylated form of Apt21-2 (PEG21-2idT) exhibited IC_{50} ranges of 1-2 nM and 70-80 nM in human and mouse cells, respectively. When administered immediately after immunization with GPI or MOG, PEG21-2idT inhibited the development of arthritic or neurological symptoms in a dose-dependent manner. Significantly, PEG21-2idT slowed the progression of arthritis when administered after the onset of GPI induced arthritis. Our findings indicate that the chemically processed anti-IL-17A aptamer, PEG21-2idT, inhibits IL-17A action and the development of autoimmunity in mouse models. These results offer for the first time an aptamer-based therapeutic approach for the treatment of Th17 cell-mediated autoimmune disorders.

2. Conformational Plasticity of RNA Aptamer against Human IgG

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We have performed a crystallographic study of RNA aptamer in complex with the Fc fragment of human IgG1. The complex has been crystallized in 20% polyethylene glycol 1000, 0.2 M calcium acetate, 100 mM Tris-HCl (pH 8.0), at 294 K by the vapor diffusion method. X-ray diffraction studies reveal that the crystals belong to the orthorhombic space group $P2_12_12$ with unit-cell parameters a = 83.7, b = 107.2, c = 79.0 Å. We have determined the 2.15 Å crystal structure of the Fc fragment of human IgG1 (hFc1) complexed with the aptamer. The aptamer adopts a distorted structure, which are stabilized by a calcium ion, explaining why the calcium ions are required for binding activity. The crystal structure demonstrates that the aptamer-hFc1 interaction involves mainly van der Waals contacts and hydrogen bonds rather than electrostatic forces. The structure also sheds light on the question of how the aptamer exhibits the high specificity to the human IgGs, not to the IgGs from other sources.

3. tRNA Mimicry in Translation Termination and mRNA Surveillance

Koichi Ito, Miki Wada, Kazuki Saito

Despite its similarities with the eukaryotic system, the molecular mechanism of translation termination in archaea remains unclear. The archaeal and eukaryotic class I release factors (RF), aRF1 and eRF1, respectively, both recognize three stop codons and catalyze polypeptide-chain release from the ribosome. In eukaryotes, eRF3, which is homologous to the tRNA carrier GTPase EF1α, forms a heterodimeric complex with eRF1 to decipher stop codons in a GTP hydrolysis dependent manner. However, genome-wide searches of archaea have failed to detect any counterparts to eRF3. Here we solved the first crystal structure of aRF1 from an archaeon, *Aeropyrum pernix* (ape-aRF1). Besides the overall tRNA mimicry, aRF1 maintains the minimal structure for binding to the eRF3 GTPase. Our biochemical and genetic analyses revealed that the authentic archaeal EF1α acts as a carrier GTPase for aRF1, and surprisingly, for aPelota, which functions in the mRNA surveillance pathways via tRNA mimicry. The versatile binding capacity of archaeal EF1α not only explains the absence of GTPase orthologs for the tRNA-mimicry proteins in archaea but also suggests that universal molecular mechanisms, which are facilitated by tRNA and tRNA mimicry proteins, underlie translational elongation and termination, and mRNA surveillance pathways.

4. Yeast Prion: [*PSI*$^+$] Aggregate Enlargement in *rnq1* Non-Prion Domain Mutants, Leading to a Loss-of-Prion in Yeast

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[*PIN*$^+$] is the prion form of the Rnq1 protein of unknown function in *Saccharomyces cerevisiae*. A glutamine (Q), asparagine (N) rich C-terminal domain is necessary for propagation of [*PIN*$^+$], while the N-terminal region is non-Q/N rich and considered the non-prion domain. Here, we isolated numerous single amino acid mutations in Rnq1, phenotypically similar to Rnq1Δ100, which inhibit [*PSI*$^+$] propagation in the [*PIN*$^+$] state, but not in the [*pin*$^-$] state, when overproduced. The dynamics of the prion aggregates was analyzed by semi-denaturing detergent-agarose gel electrophoresis and fluorescence correlation spectroscopy. The data indicated that [*PSI*$^+$] aggregates were enlarged in mother cells and, instead, not apparently transmitted into daughter cells. Under these conditions, the activity of Hsp104, a known prion disaggregase, was not affected when monitored for the thermotolerance of the *rnq1* mutants. These [*PSI*$^+$]-inhibitory *rnq1* mutations did not affect [*PIN*$^+$] propagation itself when over-expressed from a strong promoter, but instead destabilized [*PIN*$^+$] when expressed from the weak authentic *RNQ1* promoter. The majority of these mutated residues are mapped to the surface, and on one-side, of contiguous α-helices of the non-prion domain of Rnq1, suggesting its involvement in interactions with a prion or a factor necessary for prion development.

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染色体制御分野

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Our research aim is to understand various life phenomena at an atomic resolution. Our approach is to determine the tertiary structures of proteins and nucleic acids crucial for biological processes by X-ray crystallography, generate hypotheses how their functions emerge from the structures, by using computer simulation, and demonstrate the hypotheses by in vitro and in vivo functional analyses of mutants designed based on the structures. We focus on
I. Processing, chemical modification and aminoacylation of transfer RNA
II. Sensing receptors (channels) and membrane transporters
III. Structure-based cancer research.

I. Processing, chemical modification and aminoacylation of transfer RNA

Transfer RNA (tRNA) acts as an adaptor molecule to link the genetic code (in messenger RNA) to a specific amino acid. tRNA is initially transcribed by RNA polymerase as a precursor RNA with long extensions at the 5′ and 3′ terminus. Maturation of tRNA into a functional RNA requires processing of the extensional sequences, chemical modifications and specific aminoacylation. The post-transcriptional chemical modifications contribute to the structural stabilization and the specific codon recognition by tRNA. We are promoting structure determination of the tRNA-maturating enzymes in a complex with tRNA (precursor) to especially elucidate the dynamic mechanism of their highly specific chemical reactions.

1. Editing mechanism of aminoacyl-tRNA synthetases operates by a hybrid ribozyme/protein catalyst

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Aminoacyl-tRNA synthetases (aaRSs) are critical for the translational process, catalyzing the attachment of specific amino acids to their cognate tRNAs. To ensure formation of the correct aminoacyl-tRNA, and thereby enhance the reliability of translation, several aaRSs have an editing capability that hinders formation of misaminoacylated tRNAs. We investigated theoretically the mechanism of the editing reaction for a class I enzyme, leucyl-tRNA synthetase (LeuRS), complexed with a misaminoacylated tRNA(Leu), employing ab initio hybrid quantum mechanical /molecular mechanical potentials in conjunction with molecular dynamics simulations. It is shown that the water molecule that acts as the nucleophile in the editing reaction is activated by a 3′-hydroxyl group at the 3′-end of tRNA (Leu) and that the O2′ atom of the leaving group of the substrate is capped by one of the

water's hydrogen atoms. Thus, it is shown that editing is a self-cleavage reaction of the tRNA and so it is the tRNA, and not the protein, that drives the reaction. The protein does, however, have an important stabilizing effect on some high-energy intermediates along the reaction path, which is more efficient than the ribozyme would be alone. This indicates that editing is achieved by a novel "hybrid ribozyme/protein catalyst". Analysis of existing experimental data and additional modeling shows that this ribozymal mechanism appears to be widespread, occurring in the ribosome as well as in other aaRSs. It also suggests transitional forms that could have played an important role in the RNA world hypothesis for the origin of life.

2. Structure of an archaeal non-discriminating glutamyl-tRNA synthetase: a missing link in the evolution of Gln-tRNA^{Gln} formation

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The molecular basis of the genetic code relies on the specific ligation of amino acids to their cognate tRNA molecules. However, two pathways exist for the formation of Gln-tRNA(Gln). The evolutionarily older indirect route utilizes a non-discriminating glutamyl-tRNA synthetase (ND-GluRS) that can form both Glu-tRNA(Glu) and Glu-tRNA(Gln). The Glu-tRNA(Gln) is then converted to Gln-tRNA(Gln) by an amidotransferase. Since the well-characterized bacterial ND-GluRS enzymes recognize tRNA(Glu) and tRNA(Gln) with an unrelated α-helical cage domain in contrast to the β-barrel anticodon-binding domain in archaeal and eukaryotic GluRSs, the mode of tRNA(Glu)/tRNA(Gln) discrimination in archaea and eukaryotes was unknown. Here, we present the crystal structure of the Methanothermobacter thermautotrophicus ND-GluRS, which is the evolutionary predecessor of both the glutaminyl-tRNA synthetase (GlnRS) and the eukaryotic discriminating GluRS. Comparison with the previously solved structure of the Escherichia coli GlnRS-tRNA(Gln) complex reveals the structural determinants responsible for specific tRNA(Gln) recognition by GlnRS compared to promiscuous recognition of both tRNAs by the ND-GluRS. The structure also shows the amino acid recognition pocket of GluRS is more variable than that found in GlnRS. Phylogenetic analysis is used to reconstruct the key events in the evolution from indirect to direct genetic encoding of glutamine.

3. Expanding role of the jumonji C domain as an RNA hydroxylase

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JmjC (Jumonji C) domain-containing proteins are known to be an extensive family of Fe(II)/2-oxoglutarate-dependent oxygenases involved in epigenetic regulation of gene expression by catalyzing oxidative demethylation of methylated histones. We report here that a human JmjC protein named Tyw5p (TYW5) unexpectedly acts in the biosynthesis of a hypermodified nucleoside, hydroxywybutosine, in tRNA(Phe) by catalyzing hydroxylation. The finding provides an insight into the expanding role of JmjC protein as an RNA hydroxylase.

4. C-terminal domain of archaeal O-phosphoseryl-tRNA kinase displays large-scale motion to bind the 7-bp D-stem of archaeal tRNA^{Sec}

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O-Phosphoseryl-tRNA kinase (PSTK) is the key enzyme in recruiting selenocysteine (Sec) to the genetic code of archaea and eukaryotes. The enzyme phosphorylates Ser-tRNA(Sec) to produce O-phosphoseryl-tRNA(Sec) (Sep-tRNA (Sec)) that is then converted to Sec-tRNA(Sec) by Sep-tRNA:Sec-tRNA synthase. Earlier we reported the structure of the Methanocaldococcus jannaschii PSTK (MjPSTK) complexed with AMPPNP. This study presents the crystal structure (at 2.4-Å resolution) of MjPSTK complexed with an anticodon-stem/loop truncated tRNA (Sec) (Mj*tRNA(Sec)), a good enzyme substrate. Mj*tRNA(Sec) is bound between the enzyme's C-terminal domain (CTD) and N-terminal kinase domain (NTD) that are connected by a flexible 11 amino acid linker. Upon Mj*tRNA(Sec) recognition the CTD undergoes a 62-Å movement to allow proper binding of the 7-bp D-stem. This large reorganization of the PSTK quaternary structure likely provides a means by which the unique tRNA(Sec) species can be accurately recognized with high affinity by the translation

machinery. However, while the NTD recognizes the tRNA acceptor helix, shortened versions of MjPSTK (representing only 60% of the original size, in which the entire CTD, linker loop and an adjacent NTD helix are missing) are still active in vivo and in vitro, albeit with reduced activity compared to the full-length enzyme.

5. Structural Basis for mRNA Surveillance by Archaeal Pelota and GTP-bound EF1□ Complex

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No-go decay and nonstop decay are mRNA surveillance pathways that detect translational stalling and degrade the underlying mRNA, allowing the correct translation of the genetic code. In eukaryotes, the protein complex of Pelota (yeast Dom34) and Hbs1 translational GTPase recognizes the stalled ribosome containing the defective mRNA. Recently, we found that archaeal Pelota (aPelota) associates with archaeal elongation factor 1α (aEF1α) to act in the mRNA surveillance pathway, which accounts for the lack of an Hbs1 ortholog in archaea. Here we present the complex structure of aPelota and GTP-bound aEF1α determined at 2.3-Å resolution. The structure reveals how GTP-bound aEF1α recognizes aPelota and how aPelota in turn stabilizes the GTP form of aEF1α. Combined with the functional analysis in yeast, the present results provide structural insights into the molecular interaction between eukaryotic Pelota and Hbs1. Strikingly, the aPelota·aEF 1α complex structurally resembles the tRNA·EF-Tu complex bound to the ribosome. Our findings suggest that the molecular mimicry of tRNA in the distorted "A/T state" conformation by Pelota enables the complex to efficiently detect and enter the empty A site of the stalled ribosome.

6. Crystal structure of a novel JmjC-domain-containing protein, TYW5, involved in tRNA modification

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Wybutosine (yW) is a hypermodified nucleoside found in position 37 of tRNA(Phe), and is essential for correct phenylalanine codon translation. yW derivatives widely exist in eukaryotes and archaea, and their chemical structures have many species-specific variations. Among them, its hydroxylated derivative, hydroxywybutosine (OHyW), is found in eukaryotes including human, but the modification mechanism remains unknown. Recently, we identified a novel Jumonji C (JmjC)-domain-containing protein, TYW5 (tRNA yW-synthesizing enzyme 5), which forms the OHyW nucleoside by carbon hydroxylation, using Fe(II) ion and 2-oxoglutarate (2-OG) as cofactors. In this work, we present the crystal structures of human TYW 5 (hTYW5) in the free and complex forms with 2-OG and Ni(II) ion at 2.5 and 2.8 Å resolutions, respectively. The structure revealed that the catalytic domain consists of a β-jellyroll fold, a hallmark of the JmjC domains and other Fe(II)/2-OG oxygenases. hTYW5 forms a homodimer through C-terminal helix bundle formation, thereby presenting a large, positively charged patch involved in tRNA binding. A comparison with the structures of other JmjC-domain-containing proteins suggested a mechanism for substrate nucleotide recognition. Functional analyses of structure-based mutants revealed the essential Arg residues participating in tRNA recognition by TYW5. These findings extend the repertoire of the tRNA modification enzyme into the Fe(II)/2-OG oxygenase superfamily.

7. Omnipotent role of archaeal elongation factor 1 alpha (EF1α in translational elongation and termination, and quality control of protein synthesis

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The molecular mechanisms of translation termination and mRNA surveillance in archaea remain unclear. In eukaryotes, eRF3 and HBS1, which are homologous to the tRNA carrier GTPase EF1α, respectively bind eRF1 and Pelota to decipher stop codons or to facilitate mRNA surveillance. However, genome-wide searches of archaea have failed to detect any orthologs to both GTPases. Here, we report the crystal structure of aRF1 from an archaeon, Aeropyrum pernix, and present strong evidence that the authentic archaeal EF1α acts as a carrier GTPase for aRF1 and for aPelota. The binding interface residues between aRF1 and aEF1α predicted from aRF1·aEF1α·GTP ternary structure model were confirmed by in vivo functional assays. The aRF1/eRF1 structural domain with GGQ motif, which corresponds to the CCA arm of tRNA, contacts with all three structural domains of aEF1α showing striking tRNA mimicry of aRF1/eRF1 and its GTPase-mediated catalysis

for stop codon decoding. The multiple binding capacity of archaeal EF1α explains the absence of GTPase orthologs for eRF3 and HBS1 in archaea species and suggests that universal molecular mechanisms underlie translational elongation and termination, and mRNA surveillance pathways.

II. Sensing receptors (channels) and membrane transporters

Five senses (touch, taste, hearing, eyesight and smell) are essential for higher eukaryotes to determine their actions in response to environmental insults. We promote structure determination of the sensing receptors complexed with ligands to elucidate the general mechanism of how external chemical and physical stimuli activate and change the conformation of the sensing receptors (channels) to form a novel interaction with the coupled G proteins or to change the cation permeability. We are further promoting X-ray crystallography of metal transporters and membrane translocon to elucidate the fundamental mechanism of the specific substance transportation through lipid bilayer membrane. Especially, we focus on the mechanism how transporters drive their transport, how transporters discriminate their specific substrate and how transporters regulate their gating.

1. Molecular mechanisms underlying the early stage of protein translocation through the Sec translocon.

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The Sec translocon, a protein-conducting channel, consists of a heterotrimeric complex (SecYEG in bacteria and Sec61 alpha beta gamma in eukaryotes) that provides a pathway for secretary proteins to cross membranes, or for membrane proteins to integrate into the membrane. The Sec translocon alone is a passive channel, and association with channel partners, including the ribosome or SecA ATPase in bacteria, is needed for protein translocation. Three recently published crystal structures of SecY are considered to represent the closed (resting state), pre-open (transitional state determined with the bound Fab fragment mimicking SecA interaction), and SecA-bound forms. To elucidate mechanisms of transition between closed and pre-open forms, we performed all-atom molecular dynamics simulations for the pre-open form of Thermus thermophilus SecYE and the closed form of Methanococcus janaschii SecYE beta in explicit solvent and membranes. We found that the closed form of SecY is stable, while the pre-open form without the Fab fragment undergoes large conformational changes toward the closed form. The pre-open form of SecY with Fab remains unchanged, suggesting that the cytosolic interaction mimicking SecA binding stabilizes the pre-open form of SecY. Importantly, a lipid molecule at the lateral gate region appears to be required to maintain the pre-open form in the membrane. We propose that the conformational transition from closed to pre-open states of SecY upon association with SecA facilitates intercalation of phospholipids at the lateral gate, inducing initial entry of the positively charged signal peptide into the channel.

III. Structure-based cancer research

We promote X-ray crystallography of various oncogenic products or signal transduction proteins in a complex form to provide the structural basis for the mechanism of how their dysfunction cause cancer and metastasis of cancer cells. We mainly focus on growth factor receptors, oncogenic mediators and transcriptional factors, which transduce the TGF-β and Wnt/β-catenine signals. We are also promoting the project on innate immunity. Our final goal is to design a novel and effective anti-cancer drugs with minimum side effects, on the basis of their atomic structures.

1. Crystal structure of autotaxin and insight into GPCR activation by lipid mediators

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Autotaxin (ATX, also known as Enpp2) is a secreted lysophospholipase D that hydrolyzes lysophosphatidylcholine to generate lysophosphatidic acid (LPA), a lipid mediator that activates G protein-coupled receptors to evoke various cellular responses. Here, we report the crystal structures of mouse ATX alone and in complex with LPAs with different acyl-chain lengths and saturations. These structures reveal that the multidomain architecture helps to maintain the structural rigidity of the lipid-binding pocket, which accommodates the respective LPA molecules in distinct conformations. They indicate that a loop region in the catalytic domain is a major determinant for the substrate specificity of the Enpp family enzymes. Furthermore, along with biochemical and biological data, these structures suggest that the produced LPAs are

delivered from the active site to cognate G protein-coupled receptors through a hydrophobic channel.

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