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

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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.

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 down-regulates 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.

2. The temporal pattern of external stimulation determines the extent and duration of MAPK activation in a *C. elegans sensory neuron*

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Mechanisms of MAPK activation and regulation have been elucidated in such detail that it is becoming possible to computationally predict the dynamics of a MAPK signaling pathway. However, it is still difficult to actually monitor the dynamics of MAPK activation in single cells in a living organism. The conventional methods used to detect MAPK activity, such as immunostaining of fixed cells or immunoblotting of cell extracts using phospho-MAPK-specific antibodies can show only static snapshots and/or population averages of MAPK activation. Furthermore, most experimental analyses of MAPK pathways have been limited to measurement of responses to continuous or step-wise stimulation. A more sophisticated approach to measurement of MAPK pathway activation is a control-systems-engineering type of analysis that consists of application of a set of defined oscillatory inputs followed by measurement of output responses, from which system properties are deduced. In this context, sensory neurons are ideal model cells for systems analysis, as they can naturally respond to rapidly changing external stimuli.

This year, we applied a FRET-based MAPK activity probe to the *Caenorhabditis elegans* ASER sensory neuron, which is excited when environmental NaCl concentration is decreased. We exposed ASER to various cyclic patterns of stimulation, and monitored the activation dynamics of MPK-1 (an ERK-MAPK homolog). Our data demonstrated that the intensity and duration of MPK-1 activation were determined by the temporal pattern of stimulation, namely a combination of stimulation frequency, stimulation length, and inter-stimulation length. The complex, non-linear relationship between stimulation and MPK-1 activation was explained by the properties of $[Ca^{2+}]_i$ responses upstream of MPK-1.

Publications

1. Kubota, Y., O'Grady, P., Saito, H. and Takekawa, M. Oncogenic Ras abrogates MEK SUMOylation that suppresses the ERK pathway and cell transformation. *Nat. Cell Biol*. 13: 282-291, 2011.

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. Age-dependent regulation of depression-like behaviors through modulation of adrenergic receptor α_{1A} subtype expression revealed by the analysis of IL-1Ra knockout mice

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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 administration of adrenergic receptor (AR) antagonists against the ARα_1, ARα_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 ARα_{1A} subtype was significantly changed in the cerebral cortex. Interestingly, the change in expression of the ARα_{1A} subtype was correlated with an age-dependent alteration in the TST and FST in IL-1 Ra KO mice. Furthermore, mild immobilization stress loaded on C57BL/6J control mice caused similar anti-depression-like phenotypes in the TST and FST to those observed in mutant mice. These results suggest that sustained activation of IL-1 signaling induced by gene manipulation in mutant mice affects the expression of the ARα_{1A} subtype and that modification of 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.

2. 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 mechanism 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.

3. Specific regulation of spatial memory and pattern separation by plexin-A2 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 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 (PlxnA2), one of the type A plexins that mediate repulsive activities of the class 6 semaphorins, regulates the distribution of mossy fiber terminals in the CA3 region and that the mutant mice lacking PlxnA2 (*PlxnA2-/-* 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 *PlxnA2-/-* mice. We found that sensorimotor reflexes and emotional behaviors of *PlxnA2-/-* mice were normal, although motor learning and swimming abilities were 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, *PlxnA2-/-* 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 PlxnA2 may be a specific determinant of the ability of spatial reference memory and pattern separation.

4. Functional coupling of the metabotropic glutamate receptor, inositol triphosphate receptor and L-type Ca^{2+} channel in mouse CA1 pyramidal cells

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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, the voltage-dependent calcium channel (VDCC) is the major source of calcium influx, but its modulation by synaptic activity has still been under debate. While the metabotropic glutamate receptor (mGluR) is supposed to modulate L-type VDCCs (L-VDCCs), its reported actions include both facilitation and suppression, probably reflecting the uncertainty of both the molecular targets of the mGluR agonists and the source of the recorded calcium signal in previous reports. In this study, using subtype-specific knockout mice, we have shown that mGluR5 induces facilitation of the depolarization-evoked calcium current. This facilitation was not accompanied by the change in single-channel properties of the VDCC itself; instead, it required the activation of calcium-induced calcium release (CICR) that was triggered by VDCC opening, suggesting that the opening of CICR-coupled cation channels was essential for the facilitation. This facilitation was blocked or reduced by the inhibitors of both L-VDCCs and inositol triphosphate receptors (IP3Rs). Furthermore, L-VDCCs and mGluR5 were shown to form a complex by coimmunoprecipitation, suggesting that the specific functional coupling between mGluR5, IP3Rs and L-VDCCs played a pivotal role in the calcium-current facilitation. Finally, we showed that mGluR5 enhanced VDCC-dependent long-term potentiation (LTP) of synaptic transmission. Our study has identified a novel mechanism of the interaction between the mGluR and calcium signaling, and suggested contribution of mGluR5 to synaptic plasticity.

5. Dynamic development of the first synapse impinging on adult-born neurons in the olfactory bulb circuit

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The olfactory bulb (OB) receives and integrates newborn interneurons throughout life. This process is important for the proper functioning of the OB circuit and consequently, for the sense of smell. Although we know how these new interneurons are produced, the way in which they integrate into the pre-existing ongoing circuits remains poorly documented. Bearing in mind that glutamatergic inputs onto local OB interneurons are crucial for adjusting the level of bulbar inhibition, it is important to characterize when and how these inputs from excitatory synapses develop on newborn OB interneurons. We studied early synaptic events that lead to the formation and maturation of the first glutamatergic synapses on adult-born granule cells (GCs), the most abundant subtype of OB interneuron. Patch-clamp recordings and electron microscopy (EM) analysis were performed on adult-born interneurons shortly after their arrival in the adult OB circuits. We found that both the ratio of N-methyl-D-aspartate receptor (NMDAR) to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), and the number of functional release sites at proximal inputs reached a maximum during the critical period for the sensory-dependent survival of newborn cells, well before the completion of dendritic arborization. EM analysis showed an accompanying change in postsynaptic density shape during the same period of time. Interestingly, the latter morphological changes disappeared in more mature newly-formed neurons, when the NMDAR to AMPAR ratio had decreased and functional presynaptic terminals expressed only single release sites. Together, these findings show that the first glutamatergic inputs to adult-generated OB interneurons undergo a unique sequence of maturation stages.

6. Dysfunction of the RAR/RXR signaling pathway in the forebrain impairs hippocampal memory and synaptic plasticity.

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Retinoid signaling pathways mediated by retinoic acid receptor (RAR)/retinoid X receptor (RXR)-mediated transcription play critical roles in hippocampal synaptic plasticity. Furthermore, recent studies have shown that treatment with retinoic acid alleviates age-related deficits in hippocampal long-term potentiation (LTP) and memory performance and, furthermore, memory deficits in a transgenic mouse model of Alzheimer's disease. However, the roles of the RAR/RXR signaling pathway in learning and memory at the behavioral level have still not

been well characterized in the adult brain. We here show essential roles for RAR/RXR in hippocampus-dependent learning and memory. In the current study, we generated transgenic mice in which the expression of dominant-negative RAR (dnRAR) could be induced in the mature brain using a tetracycline-dependent transcription factor and examined the effects of RAR/RXR loss. The expression of dnRAR in the forebrain down-regulated the expression of RARβ, a target gene of RAR/RXR, indicating that dnRAR mice exhibit dysfunction of the RAR/RXR signaling pathway. Similar with previous findings, dnRAR mice displayed impaired LTP and AMPA receptor-mediated synaptic transmission in the hippocampus. More importantly, these mutant mice displayed impaired hippocampus-dependent social recognition and spatial memory. However, these deficits of LTP and memory performance were rescued by stronger conditioning stimulation and spaced training, respectively. Finally, we found that pharmacological blockade of RARα in the hippocampus impairs social recognition memory. From these observations, we concluded that the RAR/RXR signaling pathway greatly contributes to learning and memory, and LTP in the hippocampus in the adult brain.

Publications

Arima-Yoshida, F., Watabe, A.M. and Manabe, T. The mechanisms of the strong inhibitory modulation of long-term potentiation in the rat dentate gyrus. *Eur. J. Neurosci*. 33:1637-1646, 2011.

Wakabayashi, C., Kiyama, Y., Kunugi, H., Manabe, T. and Iwakura, Y. Age-dependent regulation of depression-like behaviors through modulation of adrenergic receptor α_{1A} subtype expression revealed by the analysis of IL-1Ra knockout mice. *Neuroscience* 192:475-484, 2011.

Katagiri, H., Pallotto, M., Nissant, A., Murray, K., Sassoè-Pognetto, M. and Lledo, P.-M. Dynamic development of the first synapse impinging on adult-born neurons in the olfactory bulb circuit. *Neural Systems & Circuits* 1:6, 2011.

Nomoto, M., Takeda, Y., Uchida, S., Mitsuda, K., Enomoto, H., Saito, K., Choi, T., Watabe, A. M., Kobayashi, S., Masushige, S., Manabe, T. and Kida, S. Dysfunction of the RAR/RXR signaling pathway in the forebrain impairs hippocampal memory and synaptic plasticity. *Molecular Brain* 5:8, 2012.

Delawary, M., Tezuka, T., Kiyama, Y., Yokoyama, K., Wada, E., Wada, K., Manabe, T., Yamamoto, T. and Nakazawa, T. NMDAR2B tyrosine phosphorylation is involved in thermal nociception. *Neurosci. Lett*. 2012 (in press).

片桐大之，真鍋俊也．長期増強・長期抑圧．Clinical Neuroscience　29巻2号．中外医学社．pp. 148-151，2011．

真鍋俊也．興奮性組織：神経 「ギャノング生理学」第23版　第Ⅱ編　神経細胞と筋肉細胞の生理学　4章(翻訳)．丸善．pp. 93-109，2011．

真鍋俊也．スライスパッチによるシナプス可塑性解析法「最新パッチクランプ実験技術法」．岡田泰伸編集．吉岡書店．pp. 103-109，2011．

Department of Basic Medical Sciences

Division of Molecular Biology, Department of Basic Medical Sciences
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. Antagonistic RNA Aptamer Specific to a Heterodimeric Form of Human interleukin-17A/F

Hironori Adachi, Akira Ishiguro, and Yoshikazu Nakamura

Interleukin-17 (IL-17) 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. The two most structurally related IL-17 family members, IL-17A and IL-17F, form homodimeric (IL-17A/A, IL-17F/F) and heterodimeric (IL-17A/F) complexes. Although the biological significance of IL-17A and IL-17F have been investigated using respective antibodies or gene knockout mice, the functional study of IL-17A/F heterodimeric form has been hampered by the lack of an inhibitory tool specific to IL-17A/F. In this study, we aimed to develop an RNA aptamer that specifically inhibits IL-17 A/F. Aptamers are short single-stranded nucleic acid sequences that are selected *in vitro* based

on their high affinity to a target molecule. One selected aptamer against human IL-17A/F, AptAF42, was isolated by repeated cycles of selection and counterselection against heterodimeric and homodimeric complexes, respectively. Thus, AptAF42 bound IL-17A/F but not IL-17A/A or IL-17F/F. The optimized derivative, AptAF42 dope1, blocked the binding of IL-17A/F, but not of IL-17A/A or IL-17F/F, to the IL-17 receptor in the surface plasmon resonance assay *in vitro*. Consistently, AptAF42dope1 blocked cytokine GRO-α production induced by IL-17A/F, but not by IL-17A/A or IL-17F/F, in human cells. An RNA footprinting assay using ribonucleases against AptAF42dope1 in the presence or absence of IL-17A/F revealed that part of the predicted secondary structure fluctuates between alternate forms and that AptAF42dope1 is globally protected from ribonuclease cleavage by IL-17A/F. These results suggest that the selected aptamer recognizes a global conformation specified by the heterodimeric surface of IL-17A/F.

2. Selection of RNA Aptamers against Mouse Embryonic Stem Cells

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Embryonic stem cells (ESCs) are capable of unlimited self-renewal and differentiation into multiple cell types. Recent large-scale analyses have identified various cell surface molecules on ESCs. Some of them are considered to be beneficial markers for characterization of cellular phenotypes and/or play an essential role for regulating the differentiation state. Thus, it is desired to efficiently produce affinity reagents specific to these molecules. In this study, to develop such reagents for mouse ESCs (mESCs), we selected RNA aptamers against intact, live mESCs using several selection strategies. The initial selection provided us with several anti-mESC aptamers of distinct sequences, which unexpectedly react with the same molecule on mESCs. To isolate aptamers against different surface markers on mESCs, one of the selected aptamers was used as a competitor in the subsequent selections. In addition, one of the selections further employed negative selection against differentiated mouse cells. Consequently, we successfully isolated three classes of anti-mESC aptamers that do not compete with one another. The isolated aptamers were shown to distinguish mESCs from differentiated mouse cell lines. In addition, the fluorescein-labeled aptamers were able to stain mESCs, but not mESC-derived differentiated cells, and the signal gradually disappeared upon induction of differentiation. These aptamers could prove useful for developing molecular probes and manipulation tools for mESCs.

3. Inhibitory RNA Aptamer against SP6 RNA Polymerase

Yusuke Mori, Shoji P. Ohuchi, and Yoshikazu Nakamura

Aptamers are attractive tools for modulating function of a desired target. In this study, we isolated an RNA aptamer which specifically inhibits transcription of SP6 RNA polymerase. The dissociation constant and 50% inhibitory concentration of the aptamer were estimated as 9.5 nM and 24.8 nM, respectively. Biochemical analyses revealed that the aptamer adopts the structure including two stems, two loops, and 5′ single-stranded region. Based on the results, the aptamer could be engineered to circular permutant and binary construct forms without decreasing the activity. The aptamer would be applicable for the construction of expression regulation systems.

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

Hiroshi Kurahashi, Chan-Gi Pack[1], Shoichiro Shibata, Keita Oishi, Yasushi Sako[1], and Yoshikazu Nakamura: [1]Cellular Informatics Laboratory, RIKEN Advanced Science Institute

[*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.

5. Yeast Prion Curing by Lsm4, a Triggering Factor for Processing Body Assembly in *Saccharomyces cerevisiae*

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Prions are epigenetic modifiers that cause partially loss-of-function of proteins containing, mostly but not all, glutamine(Q)/asparagine(N)-rich domains by forming transmissible protein amyloids in *Saccharomyces cerevisiae*. In contrast to the cellular factors and processes involved in prion induction and propagation, those for prion curing and its physiological meaning, if any, remain unknown. We have searched for yeast proteins whose overproduction cures prions. Here we report that overproduced Lsm4 eliminates three major prions [*PSI*$^+$], [*URE3*] and [*RNQ*$^+$] in yeast. Lsm4 is a component of cytoplasmic RNA granules called processing bodies (P-bodies), and plays a crucial role in P-body assembly. The Q/N-rich domain of Lsm4 is responsible for prion curing as is true for P-body assembly. Overproduced Lsm4 leads to outgrowth of prion amyloids, insusceptible to prion transmission to daughter cells, due to the interplay of Lsm4 and pre-existing prion amyloids. The fluorescence microscopic analysis and the co-immunoprecipitation experiment revealed that overproduced Lsm4 interacts with Ure3 and Sup35 in the prion state, suggesting that outgrowth of prion amyloids is induced by abnormal Lsm4 contact with pre-existing prions.

Publications

1 Ishiguro, A., Akiyama, T., Adachi, H., Inoue, J., Nakamura, Y.: Therapeutic potential of anti-interleukin-17A aptamer: suppression of IL-17A signaling and attenuation of autoimmunity in mouse models. Arthritis Rheum., 63: 455-466 (2011).

2 Kurahashi, H., Pack, C., Shibata, S., Oishi, K., Sako, Y., Nakamura, Y.: [*PSI*$^+$] aggregate enlargement in *rnq1* non-prion domain mutants, leading to a loss-of-prion in yeast. Genes Cells, 16: 576-589 (2011).

3 Nakamura, Y., Ito, K.: tRNA mimicry in translation termination and beyond. Wiley Interdiscip. Rev. RNA, 2: 647-668 (Published Online March 30, 2011) (2012).

4 Adachi, H., Ishiguro, A., Hamada, M., Sakota, E., Asai, K., Nakamura, Y.: Antagonistic RNA aptamer specific to a heterodimeric form of human interleukin-17A/F. Biochimie, 93: 1081-1088 (2011).

5 Iwagawa, T., Ohuchi, S.P., Watanabe, S., Nakamura, Y.: Selection of RNA aptamers against mouse embryonic stem cells. Biochimie, 94: 250-257 (2011).

6 Kurahashi, H., Oishi, K., Nakamura, Y.: A bipolar personality of yeast prion proteins, Prion, 5: 305-310 (2011).

7 Nakamura, Y., Ishiguro, A., Miyakawa, S.: RNA plasticity and selectivity applicable to therapeutics and novel biosensor development. Genes to Cells, in press.

8 Fujimoto, Y., Nakamura, Y., Ohuchi, S.P.: HEXIM1 binding elements on mRNAs identified through transcriptomic SELEX and computational screening. Biochimie, in press.

9 Mori, Y., Nakamura, Y., Ohuchi, S.P.: Inhibitory RNA aptamer against SP6 RNA polymerase. Biochem. Biophys. Res. Commun., in press.

10 Nakamura, Y.: Aptamer: Biology to Applications. Advances in Polymer Science, Review Volume “Nucleic Acid Drugs”, in press.