2P031 線虫モデルを利用した神経変性疾患における病態伝播のメカニズム解明**A worm model describing propagation of protein aggregates in neurodegenerative diseases****Mariko Ogawa**¹, Hisashi Shidara², Kotaro Oka², Yoshiaki Furukawa¹ (¹*Dept. of Chem., Keio Univ.*, ²*Dept. of Biosci. Informatics, Keio Univ.*)

Fibrils caused by protein misfolding have the tendency to propagate inter/intracellularly, a mechanism known as “seeding”. Here, we investigated the seeding mechanism of Cu,Zn-superoxide dismutase (SOD1), which is known to trigger amyotrophic lateral sclerosis (ALS) when mutation occurs in the gene. An ALS-causing mutant, SOD1L126X, was used to test whether it forms aggregates in vitro with the addition of seeds. We confirmed that adding insoluble seeds to its soluble counterpart accelerates aggregation process in vitro. Also, we are currently using *C. elegans* to further understand the seeding reaction of SOD1 in a living organism. Worms expressing mutant SOD1 will become a new model of seeded aggregation in vivo.

2P032 筋萎縮性側索硬化症に関わる SOD1 タンパク質の四次構造変化を検出できるペプチドの開発**Aberrant monomer-dimer equilibrium of mutant SOD1 in ALS: Development of peptides probing protein quaternary structures****Takao Nomura**, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Cu,Zn-superoxide dismutase (SOD1) protects aerobic organisms from superoxide by catalyzing its disproportionation but gains toxic properties by mutations identified in a familial form of amyotrophic lateral sclerosis. Pathogenic mutations have been considered to facilitate the monomerization of SOD1, which usually forms a tight homo-dimer. It is, however, difficult to clarify effects of mutations on the quaternary structure of SOD1 *in vivo/ex vivo*. Here, we have developed peptides discriminating monomeric and dimeric SOD1 by using a phage display method. The peptides were found to bind exclusively to either a monomeric or dimeric state of SOD1 with a sub-micromolar dissociation constant, enabling a pull-down assay to evaluate the SOD1 quaternary structures.

2P033 SOD1 への細胞内銅イオン輸送を制御するタンパク質ネットワーク**A protein network regulating an intracellular copper transfer to superoxide dismutase****Kenta Nakagome**, Yasushi Mitomi, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Cu,Zn-superoxide dismutase (SOD1) binds a catalytic copper ion for its antioxidant activity, which is delivered by a copper chaperone for SOD1 (CCS) *in vivo*. CCS is composed of three domains, among which central and C-terminal domains play critical roles in recognition and activation of apo-SOD1, respectively. In contrast, physiological roles of the N-terminal domain (CCS^{nt}) remain obscure. Given that CCS^{nt} possesses a ferredoxin-like fold with a CxxC motif for binding a cuprous ion, we suspected if CCS^{nt} functions as a receptor site for a copper ion and thus involves in the activation of SOD1. Here we have found a certain ferredoxin-like protein that appears to exchange a copper ion with CCS^{nt}, suggesting a new intracellular pathway for copper supply to SOD1.

2P034 亜鉛イオンが制御する銅シャペロンシステムの分子認識メカニズム**Zinc ion regulates molecular recognition in copper chaperone system****Yuma Wakahara**, Kazuki Honda, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Cu,Zn-superoxide dismutase (SOD1) catalyzes the disproportionation of superoxide radicals at its bound copper ion, which is supplied by a copper chaperone for SOD1 (CCS) *in vivo*. CCS is composed of three domains, among which the central domain (CCS^{cd}) plays critical roles in recognizing the substrate, apo-SOD1. CCS^{cd} assumes a similar structural fold to that of SOD1 and, more interestingly, possesses a binding site for a zinc ion. Here we show that CCS^{cd} is required to bind a zinc ion for specific recognition of apo-SOD1. Zinc binding exerts a conformational change in CCS^{cd}, which enables to form a tight complex with apo-SOD1. It is hence possible that *in vivo* transfer of a copper ion from CCS to SOD1 is regulated by a heterogeneous metal ion, *i.e.* zinc ion.

2P035 筋萎縮性側索硬化症に関わる変異型 SOD1 タンパク質のオリゴマー化メカニズム**Oligomerization mechanism of mutant SOD1 proteins in a familial form of amyotrophic lateral sclerosis****Itsuki Anzai**, Keisuke Toichi, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Dominant mutations in Cu,Zn-superoxide dismutase (SOD1) cause a familial form of amyotrophic lateral sclerosis (fALS). Wild-type SOD1 forms an intramolecular disulfide, while pathological inclusions contain mutant SOD1 oligomers cross-linked via disulfides. Recently, we have shown that isomerization from an “intra”- to an “inter”-molecular disulfide bond is facilitated by destabilization of SOD1 by mutations. Based upon our proposing mechanism, we attempted to develop anti-oligomerization drugs effective to mutant SOD1, which may be a cure for SOD1-fALS. Several drugs screened from a drug library have been found to inhibit SOD1 oligomerization by structural stabilization of SOD1. Detailed examination on interactions of those drugs with SOD1 is now underway.

2P036 神経変性疾患における老化の役割を検証する酵母モデルの構築**A yeast model for testing roles of aging process in neurodegenerative diseases****Yuko Nishiura**, Yoshiaki Furukawa (*Dept. of Chem., Keio Univ.*)

Most of neurodegenerative diseases are characterized by its late onset and specific deterioration of a nervous system. Damages would accumulate in non-proliferating neurons with aging and then exceed a certain threshold to cause neurodegeneration. Pathological roles of aging in neurodegenerative diseases, however, remain obscure partly due to its time-consuming aspect. Here, we have attempted to reveal effects of aging on pathologies of amyotrophic lateral sclerosis by using the budding yeast, *S.cerevisiae*, as a model organism. The chronological aging of yeast as a survival of its non-dividing state can be a convenient model for the aging process in post-mitotic cells. Biochemical changes of pathogenic proteins by aging will be discussed.