membrane. Although several models for the opening process have been proposed based on molecular dynamics (MD) simulations, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. We performed MD simulations for the mechano-gating of MscL embedded in the lipid bilayer. Upon tension generation in the bilayer, Phe78 in the outer helix was dragged by lipids, leading to a tilting of the helices. Among amino acids in the outer helix facing the bilayer, Phe78 at the water-lipid interface showed the strongest interaction with lipids, thus may work as a major tension sensor. Neighboring inner helices cross each other in the inner leaflet, forming the most constricted part of the pore. In the closed state of MscL, Leu19 and Val23 in the constricted part form a stable hydrophobic environment. As tension increases, the crossings move toward the cytoplasm associated with an expansion of the constricted part and the hydrophobic environment was broken followed by water penetration and permeation. It seemed that water penetration and permeation accelerated the pore opening probably by decreasing the hydrophobic interaction between Leu19 and Val23. To support this idea, we performed MD simulations of the GOF mutant G22N with almost the same pore size, and found that G22N mutant MscL permeated water molecules without tension in the bilayer.

3P-088 FGF23 分泌における ppGalNAc-T3 の役割 Role of Polypeptide GalNAc-transferase T3 in FGF23 secretion

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Mutations in the glycosyltransferase, polypeptide GalNAc-T3 gene, involved in initiation of O-glycosylation, were recently identified as a cause of the rare autosomal recessive metabolic disorder familial tumoral calcinosis (FTC)(OMIM 211900). FTC is associated with hyperphosphatemia and massive ectopic calcifications. Here, we demonstrate that the secretion of the phosphaturic factor FGF23 requires O-glycosylation, and that GalNAc-T3 selectively directs O-glycosylation in a subtilisin-like proprotein convertase recognition sequence motif, which blocks processing of FGF23. Furthermore, this O-glycosylation is depending on the lectin domain of GalNAc-T3.

The study suggests a novel posttranslational regulatory model of FGF23 involving competing O-glycosylation and protease processing to produce intact FGF23.

3P-089 Visualization of the assembly and disassembly of *S. cerevisiae* Rad51 on duplex DNA in real-time

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DNA double-strand breaks (DSB) are the most dangerous type of DNA damage. Homologous recombination (HR) is the most reliable method to repair DSBs. The process of HR is separated into three phases, in which a RecA-like DNA strand exchange protein plays a central role. First, the end of broken DNA is processed to generate 3'-ended single-strand DNA (ssDNA) tail to which the DNA strand exchange protein binds. Second, the protein-DNA complex searches homology on a donor double-strand DNA (dsDNA) and catalyzes the pairing and exchange of DNA strands. Finally, the repaired DNA molecules are resolved.

In *S. cerevisiae*, HR is catalyzed by a RecA-like protein, Rad51. Rad51 forms a helical filament in an ATP dependent manner. In the filament, Rad51 occupies 3-4 nucleotides and stretches DNA to 150% of its B-form length. Interestingly, Rad51 binds to dsDNA with a similar affinity as for ssDNA, even though the functional form of Rad51 requires assembly on ssDNA. Since the Rad51 filament formation on dsDNA inhibits HR, the turnover of Rad51 on dsDNA is stimulated by a dsDNA translocase, Rad54.

In this study, we use single molecule fluorescence microscopy to observe the assembly and disassembly of Rad51 filament on dsDNA in real-time. We show that the Rad51 filament is stable in the presence of nucleotide cofactors and that Rad51 can hydrolyze multiple ATP molecules while remaining bound to DNA.

3P-090 バクテリオファージ Mu の標的免疫反応の光学顕微鏡下での直接観察 Direct Observation of Bacteriophage Mu Transposition Target Immunity

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DNA transposons integrate into the chromosome of their host organisms at many sites with limited target sequence specificity. In the absence of a mechanism to avoid insertion into itself, transposons would risk self-destruction. Some transposons can avoid this problem, and transposition immunity of phage Mu is

one example. MuA transposase and MuB ATP-dependent DNA-binding protein participate in this process. MuB-bound DNA is an efficient target for Mu insertion. MuA bound to the Mu DNA ends stimulates the MuB ATPase and dissociates MuB from its neighborhood, making the region near Mu ends a poor target. The stable MuA tetramer in the Mu transpososome efficiently disassembles MuB from DNA by forming DNA-loops between the transpososome and the MuB bound DNA site. We show that the average observable DNA loop size expands as the duration in which the DNA Brownian motion for loop formation is extended. The transpososome dissociate MuB molecules first from nearby DNA by forming smaller DNA loops and as the nearby MuB becomes scarce, finds MuB oligomers further away to form larger DNA loops. We propose a model in which several rounds of DNA loop formation/dissociation cycles results in translocation of the transpososome away from the transposon ends prior to insertion into distant target DNA sites.

3P-091 SUMO 化による転写調節因子 heat shock factor 2 の DNA 結合阻害の分子機構 Molecular basis for SUMOylation-dependent regulation of DNA binding activity of Heat Shock Factor 2

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The heat shock response is a cellular defense against environmental and physiological stresses, such as heat, heavy metals, oxygen radicals and viral or bacterial infections. Eukaryotic cells maintain homeostasis through elevating the expression of heat shock proteins (HSPs), which function as molecular chaperones, in response to various stress stimuli. The heat shock response is also evoked during non-stress-induced processes, such as early development and cellular differentiation in mammals. Heat shock factor 2 (HSF2) is a member of a vertebrate transcription factor family for genes of HSPs and is involved in the regulation of development and cellular differentiation. The DNA binding property of HSF2 is modulated by the post-translational modification of a specific lysine residue in its DNA binding domain by small ubiquitin-like modifier (SUMO). However the consequences of SUMOylation and its underlying molecular mechanism remain unclear. Here we show the inhibitory effect of SUMOylation on the interaction between HSF2 and DNA based on biochemical analysis using isolated recombinant HSF2. NMR study of the SUMOylated DNA binding domain of HSF2 indicates that the SUMO moiety is flexible with respect to the DNA binding domain and has neither a noncovalent interface with nor a structural effect on the domain. Combined with data from double electron-electron resonance and paramagnetic NMR relaxation enhancement experiments, these results suggest that SUMO attachment negatively modulates the formation of the protein-DNA complex through a randomly distributed steric interference.

3P-092 部位特異的変異体を用いた bHLH 因子の解析 Analyses of a bHLH transcription factor using site-directed mutants

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Basic helix-loop-helix (bHLH) transcription factors play a role in regulation of transcription. They form a homo-dimer or a hetero-dimer with other bHLH factors, and that control the transcription of genes by binding to a specific DNA sequence, E-box. However, the dynamic processes of their dimerization and DNA binding remains to be elucidated. To understand these processes, we expressed GFP-fusion protein (HEB-GFP) of a bHLH transcription factor, HEB protein (Hela E-box-binding protein) that expressed ubiquitously in most cell types of many vertebrates. When HEB-GFP was mixed with the Cy5-labeled oligonucleotides containing the E-box sequence (Cy5-Eoligo), the fluorescence resonance energy transfer (FRET) was observed, suggesting that HEB-GFP bound to Cy5-Eoligo similar to native HEB. However, HEB-GFP formed dimers even in the absence of Eoligo at low temperature unlike recombinant HEB. Dissociation constant (Kd) obtained from FRET analysis was about 9 nM for Eoligo, which shows a good agreement with those obtained from other methods (for example gel-shift assay). Then, we prepared site-directed mutants of HEB-GFP in which the 9th amino acid, a glutamic acid (Eb9), in basic region of HEB were replaced into glycine, glutamine and aspartic acid. These mutant HEB-GFPs bound Eoligo with higher Kd (19-24 nM). It was suggested that Eb9 plays a crucial role for recognition of E-box sequence.

3P-093 Leucyl-tRNA 合成酵素における酵素機能発現の計算科学的解析 Computational analyses of expression mechanisms of enzymatic functions in leucyl-tRNA synthetase

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Aminoacyl-tRNA synthetases (aaRSs) catalyze the attachment of their cognate amino acid to the 3'-end of the specific tRNAs; their strict discrimination of the cognate amino acids from non-cognate ones is crucial for the fidelity of translation. However, in some cases, e.g., the leucine system, due to existence of