S2d1-2
Signal Relay within the Membrane: Three Residues Convert a Proton Pump into a Sensory Receptor
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Microbial rhodopsins are photochemically reactive membrane-embedded proteins. They are widespread in the microbial world. A striking characteristic of these proteins is their wide range of seemingly dissimilar functions. Some are light-driven transporters, such as the proton pumps bacteriorhodopsin (BR) and proteorhodopsin. Others are light sensors, such as the phototaxis receptors sensory rhodopsin I and II (SRI and SRII). These sensory rhodopsins relay signals by protein-protein interaction to integral membrane transducer proteins HtrI and HtrII, respectively. Here we show that three mutations in BR: two on its surface (mimicking Thr189 and Tyr199 in SRII) and one bridging the retinal attachment site and the membrane-embedded surface (mimicking Thr204 in SRII), enable it to bind to the HtrII and relay the retinal photoisomerization signal to induce HtrII-mediated phototaxis responses. The results identify the minimal core mechanism of signal relay from the SRII receptor to the HtrII transducer, which entails initial storage of energy of photoisomerization in the hydrogen-bond between Thr204 and Tyr174, which is in van der Waals contact with the retinal, followed by transfer of this chemical energy to drive structural transitions in the transducer helices. The results furthermore demonstrate that evolution accomplished an elegant but simple conversion: the essential residue differences between transport and signaling proteins in the rhodopsin family are far less than previously imagined.

S2d1-3
Light-induced interaction changes of pharaonis phorobodopsin-pHtrII complex studied by FT-IR spectroscopy
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Phorobodopsin (ppR) acts as a receptor for negative photoactivation in N. pharaoonis and forms a 2:2 complex with its transducer protein pHtrII in membranes. ppR maximally absorbs green light (500 nm) by all-trans retinal chromophore and its photo-isomerization is a structural changes of ppR and the interaction changes of pHtrII. Such structural changes finally activate the phosphorylation cascades that modulate the flagella motors. By use of FTIR spectroscopy, we have studied the structural changes of ppR and pHtrII since 2001. In 2003, we found that the hydrogen-bonding alteration of Thr204 in ppR upon the retinal isomerisation is specific for the ppR/pHtrII complex and not observed in the ppR without pHtrII. In 2005, we found that the inter-molecular hydrogen bonds between Tyr199 in ppR and Asn74 in pHtrII is strengthened in the M intermediate. Recently, we measured the ppR, minus ppR spectra at 250-293 K. Significant temperature dependence was observed for the amide-I vibrations of helices of ppR only in the ppR/pHtrII complex spectra, where the amplitude was restored at room temperature. Such temperature dependence was diminished for the complex of ppR with the G83C and G83F mutants of pHtrII, which are known to abolish the photo-sensory function. It implies that G83 plays an important structural role in the activation processes of the ppR/pHtrII complex. In the symposium, I will present the recent trial for detecting the protein-protein interaction in more physiological condition as well.

S2d1-4
The photo-sensory transduction of Anabaena sensory rhodopsin with tetramer of 14kDa soluble transducer
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Microbial rhodopsins, photoactive seven transmembrane proteins, contain all trans-13-cis retinal as a chromophore. The genome sequencing projects (www.kazusa.or.jp) discovered archaean type (TypeI) opsin homologues in cyanobacteria. The Anabaena sensory rhodopsin (ASR) shows a green light-absorbing pigment (absorption max = 550 nm) in the presence of all-trans retinal and the mutation of retinal binding pocket (P206D & E) shifts the absorption maximum to the blue. ASR serves as a photoreceptor for complementary chromatic adaptation and it is proved by proteomics analysis and EMSA. The proton acceptor of ASR is not D75 (D85 in BR) but D217 in the cytoplasmic portion of ASR. In addition, the 14 kDa transducer binds to cytoplasmic part of ASR and more efficiently in the presence of 32 residues at the c-terminal of ASR. In gel shift assay, tetramer of the 14 kDa soluble protein binds the promoter of phycocyanin, phycocerythrocyanin, kai and ASR operon and shows a Protein-DNA complex labeled with radioisotope on the gel in vivo and in vitro. Therefore, ASR functions as a photoreceptor that monitors the quality of light and transmitted the information into cytoplasmic region through 14 kDa transducer which binds and regulates a several promoters involved in chromatic adaptation. This sensory transduction mechanism of ASR is totally different from the halobacterial phototaxis signal transduction which is relaying the signal through membrane-bound transducer and using two component regulatory system.

S2d1-5
Photolysis Pathway of Meta II
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Light absorption of rhodopsins chromophore 11-cis-retinal causes photoisomerization to all-trans-retinal (first switch), triggering further conformational changes of the apoprotein which subsequently proceeds through inactive intermediates such as metarhodopsin I (Meta I). Meta I is in a pH and temperature dependent equilibrium with the active metarhodopsin II (Meta II). Prerequisites for formation of Meta II are Schiff base deprotonation and uptake of a second proton. Schiff base deprotonation can only occur when the respective proton donor and proton acceptor groups are correctly adjusted. The appearance of the deprotonated form is directly connected with an increase of activity. Although blue light illumination of Meta II induces deactivating conformational changes of the receptor (second switch) , the ground state is not restored by trans/cis isomerization of the polypeptide chain. Instead, light-sensitive metarhodopsin III (Meta III) is formed by syn/anti isomerization of the Schiff base. Compared to the activating pathway, time resolved UV/Vis spectra reflecting the protonation state of the Schiff base indicate faster kinetics for the deactivation process. However, time resolved FTIR difference spectra reveal that this applies only for the isomerization and proton transfer processes of the Schiff base, whereas other deactivating conformational changes of the protein appear on a slower timescale. This allows us to deduce a more detailed model for the light induced deactivation process.