NblS/Hik33 associates to RC47 and senses stressed condition of PSII in cyanobacteria.

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Two Component System (TCS) is the common mechanism for sensing environmental change in bacteria and mainly composed by sensor Histidine Kinase (HK) and transcriptional factor Response Regulator (RR). Cyanobacteria have diverged morphology, physiology and also TCSs as reflecting their habitats. However, the NblS/Hik33-RpaB orthologs are highly conserved and essential among cyanobacteria as well as chloroplasts of rhodophyte and glaucocystophyte (the ortholog named as Ycf26-Ycf27 in chloroplasts). Because photosynthetic genes and CAB/ELIP/HLIP superfamily protein encoding genes are regulated by RpaB, this TCS is considered as the important system for maintenance of photosynthesis. Previous dedicated research suggests that NblS/Hik33 responds to multiple stresses such as high light, low temperature, high salt, high osmolarity and reactive oxygen species, however, how the single protein kinase can sense and respond to such divergent stresses remains unknown, and I analyzed the underlying mechanism for NblS of *Synechococcus elongatus* PCC 7942.

For analysis, I constructed C-terminal FLAG-tagged strain in native genomic locus of *nblS*. Cell fractionation analysis showed that NblS localized in thylakoid membrane. NblS migrated as about 400 kDa and 800 kDa complexes in clear native PAGE analysis and co-precipitated with D2 and CP47 proteins of photosystem II (PSII) in an immunoprecipitation experiment after protein cross-linking. On the other hand, CP43 was not co-precipitated in the immunoprecipitation by NblS and NblS was not detected in purified PSII complexes thorough the purification by histidine-tagged CP43 strain. These results suggested that NblS was associated with the previously identified RC47, an assembly intermediate complex of PSII. Since, it is reported that RC47 exists as monomer and dimer form, the NblS complexes observed in CN-PAGE are likely as [RC47-$NblS_2$] or [RC47-$NblS_2$]$_2$.

Take into account the previous research that NblS mediated gene expression was induced by a PSII electron transport inhibitor DCMU, it was expected that NblS signaling is triggered via PSII redox state and possible to be quenched with typical PSII alternative electron acceptors (AEAs). As expected, high light stress induced expression of *hliA*, one of HLIP encoding genes, was alleviated by AEAs. To further confirm the quenching effect, especially 2,6-dichloro-1,4-benzoquinone (DCBQ) among these AEAs, which has high affinity around Q_A site inside PSII, was applied for the other stress conditions and it was found that DCBQ quenched all responses.

From these results, I propose that NblS composes the signaling complex (NblS Sensory Complex: NSC) through direct interaction with RC47 and redox status of Q_A in RC47 is sensed as a signal to modulate the downstream gene expression.