Missing piece of two-component signal transduction systems unveiled by SEC-SAXS

To adapt to changes in the chemical and physical factors of their environment, all organisms have signal transduction systems that sense each environmental factor. In this study, we focused on a signal transduction system of root nodule bacteria (rhizobia) coexisting with legumes, which senses oxygen (O_2) levels in the soil.

The rhizobia mediate nitrogen fixation, which converts nitrogen (N_2) in the atmosphere into ammonia (NH_3), which is nitrogen nutrition available for plants. Although NH_3 in chemical fertilizers is industrially produced at a very high pressure and temperature, the rhizobia can generate ammonia at an ordinary temperature and pressure by their nitrogen fixation reaction. This reaction is catalyzed by nitrogen fixation enzymes, but these enzymes cannot function in the presence of O_2 owing to their lability to O_2. Therefore, the rhizobia have an O_2-sensing protein system, in which FixL functions as an oxygen sensor and FixJ controls the biosynthesis of nitrogen fixation enzymes in response to the O_2 concentration sensed by FixL (Fig. 1) [1], resulting in the synthesis of nitrogen fixation enzymes in anaerobic environments. The FixL/FixJ system is a so-called "two-component signal transduction system (TCS)" that consists of two types of proteins [2]. Since TCSs are ubiquitous in all living systems, except for animals including humans, it has attracted increasing attention as a development target of antimicrobial agents and plant growth promoters without side effects in animals. From this background, although numerous researchers have studied TCSs with interest for many years, it has been impossible to clarify the molecular mechanism of how living organisms sense and adapt to environmental factors in detail. This is mainly because the whole structures of TCS proteins have not yet been elucidated.

We aimed to clarify the detailed molecular mechanism of the signal transduction system involved in sensing environmental factors by elucidating the overall structure of O_2-sensing FixL/FixJ protein systems.

In this study, the structures of full-length FixL and FixJ proteins were determined by small-angle X-ray scattering (SAXS) and X-ray crystallography. To obtain accurate SAXS data, we established new equipment, SEC-SAXS, at SPring-8 **BL45XU** [3], in which size-exclusion column chromatography (SEC) equipment for protein purification and an SAXS measurement system are assembled. The system makes it possible to measure the SAXS of a fresh protein sample free from any protein aggregation, immediately after elution from a column has been enabled. Such a combined measurement system was recently installed in the synchrotron radiation facilities of the Asia-Oceania countries, although it has already been introduced in Western countries.

Figure 2 shows the newly unveiled three-dimensional structures of FixL and FixL-FixJ complexes determined by the SEC-SAXS method and X-ray crystal structure analysis at SPring-8 **BL26B2** [4,5]. These analyses provided some novel insights into the structure. FixL forms an intertwined homodimer, and there was no significant difference between the overall structures of O_2-binding and O_2-unbinding to the heme of FixL. Therefore, it is suggested that the intramolecular signal transduction in FixL caused by O_2 sensing is propagated by local structural changes (Fig. 3). For the FixL-FixJ complex, it was also found that only the receiver domain of a phosphate group in FixJ interacts with the FixL, and another domain is flexible without interacting with the FixL. Because phosphate transfer is a common function for all TCSs, the interaction between the FixL

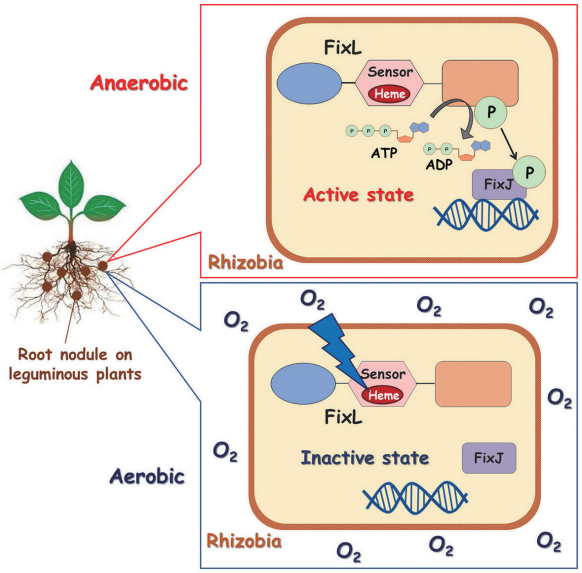

Fig. 1. O_2-sensing protein system FixL/FixJ in rhizobia. O_2 is sensed in rhizobia by a heme molecule in the sensor domain of FixL. Under an anaerobic condition, FixL does not bind O_2, and a phosphate group is generated by ATP hydrolyzation. The phosphate group is transferred from FixL to FixJ. The phosphorylated FixJ acts as a transcriptional factor for the biosynthesis of nitrogen fixation enzymes. Under an aerobic condition, O_2 is sensed by the heme molecule in the FixL, and FixJ does not act as transcriptional factor.

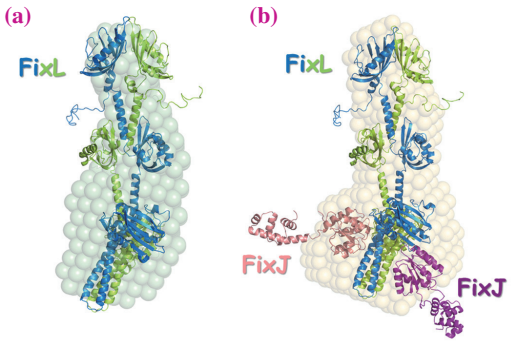

Fig. 2. Structure of the full-length FixL **(a)** and its complex with the full-length FixJ. **(b)** As revealed in this study, FixL forms a homodimer, shown as blue and green ribbons. FixJ is shown in pink and magenta.

and FixJ in this study is considered to be a common characteristic of TCS proteins. In addition, in the other proteins belonging to TCSs, protein domains with various physiological functions are fused to the domain corresponding to the flexible structure. Therefore, it is considered that TCS proteins became able to cope with various environmental factors by diversifying this flexible domain during the process of evolution.

FixL/FixJ is indispensable for the supply of nitrogen nutrients essential to the growth of soybeans, the host plant. Soybeans are a highly nutritious and useful plant, as reflected in its scientific name *Glycine max* (meaning that glycine, a kind of amino acid, is maximum). *Bradyrhizobium japonicum* solution is sprayed onto soybean seed stock at an industrial scale. Our results may open the path for genetic modification of this rhizobial TCS to improve crop yields.

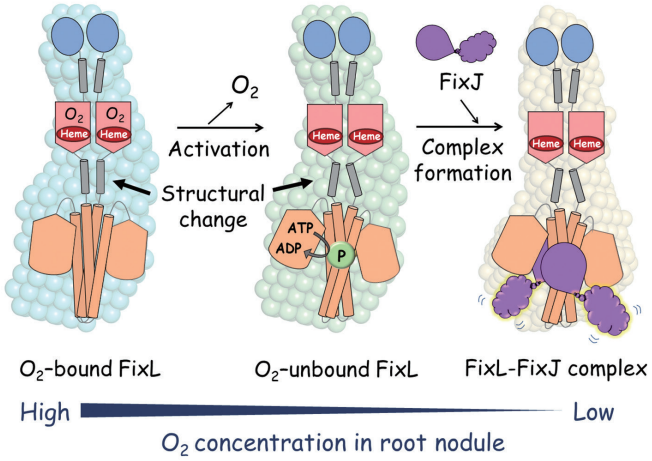

Fig. 3. Schematic diagram of the molecular mechanism of the O_2-sensing FixL/FixJ system. Our results suggest that there are no large changes in the overall structure of the full-length FixL upon O_2 dissociation from the heme. However, the orientation of the coiled-coil helices between the heme-containing sensor domain (pink) and the histidine kinase domain (orange) may change. Such a localized structural change could alter the distance between the ATP-binding site and a phosphate receiving site in the histidine kinase domain. At a low O_2 concentration, FixL and FixJ form a complex, and a phosphorylation site of FixJ approaches the phosphorylated site of FixL, which mediates phosphotransfer.

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