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Understanding the overall architecture of proteins that supply nitrogen nutrients to plants by sensing oxygen concentration in soil.

Root nodules occur on the roots of leguminous plants such as soybean, pea, and so on. The nodules associate with symbiotic bacteria known as rhizobia [1]. The rhizobia are responsible for an important reaction, called “nitrogen fixation” [1], that converts nitrogen N_2 in the Earth’s atmosphere into the ammonia NH_3, readily available for plants. Since this reaction cannot take place in the presence of oxygen, the rhizobia have a protein system that senses the oxygen concentration. The international collaborative research team led by Dr. Hitomi Sawai (University of Hyogo, Japan) and Prof. S. Samar Hasnain (University of Liverpool, United Kingdom) has elucidated the first overall structure of the protein system by using the synchrotron radiation facilities [2] “SOLEIL” in France and “SPring-8” in Japan. The results of this study will be released on April 10, 2018 (2:00 p.m. EST.) in the international scientific journal “Science Signaling” published by the American Association for the Advancement of Science (AAAS).

Background of research:

We, humans, are sensitive to the changes of environmental factors, such as oxygen, light, and heat. To adapt to these changes, humans have signal transduction systems that sense each environmental factor and maintain our lives by responding to them. Such kind of signal transduction systems present not only in humans but also in all living organisms, and each organism maintains its life by using own systems. In this study, we focused on a system that senses the concentration of oxygen in the soil by the root nodule bacteria (rhizobia) that coexist with legumes. The rhizobia mediate the reaction (nitrogen fixation), which converts nitrogen in the atmosphere into nitrogen nutrition (ammonia) available for plants. Although the ammonia contained in the chemical fertilizer is industrially produced at a high pressure and temperature (1,000 atm and 500℃), the rhizobia can generate ammonia under ordinary temperature and pressure by the reaction of nitrogen fixation. The nitrogen fixation reaction by rhizobia is catalyzed by an enzyme called nitrogenase [3], but this enzyme cannot function in the presence of oxygen. Therefore, the rhizobia have a protein system that can efficiently conduct nitrogen fixation by sensing the surrounding oxygen concentrations and synthesizing nitrogen fixation enzymes in anaerobic environments. In this system, a protein that functions as an oxygen sensor is FixL, and a protein that controls the biosynthesis of nitrogen fixation enzymes in response to oxygen sensing by FixL is FixJ (Fig. 1). The signal transduction system

that consists of two kinds of proteins, such as the FixL/FixJ system, is called the “two-component signal transduction system [4]”. The two-component signal transduction system was first identified more than 30 years ago where microorganisms and higher plants universally possess. More than 65,000 systems have been identified to date. Since the two-component signal transmission system does not exist in animals including humans, it has gain increasing attraction as a development target of antimicrobial agents and plant growth promoters without side effects to animals. From this background, even though the two-component signal transmission system is an object that many researchers have studied with interest for many years, the whole structure of the sensing protein has not been elucidated in any system. Thus, it has been impossible to clarify molecular mechanism of “how do we sense and adapt to environmental factors?” in detail.

Our research group aimed to clarify the detailed molecular mechanism on the signal transduction system involving sensing environmental factors by elucidating overall structure of oxygen-sensing two-component FixL/FixJ proteins in rhizobia.

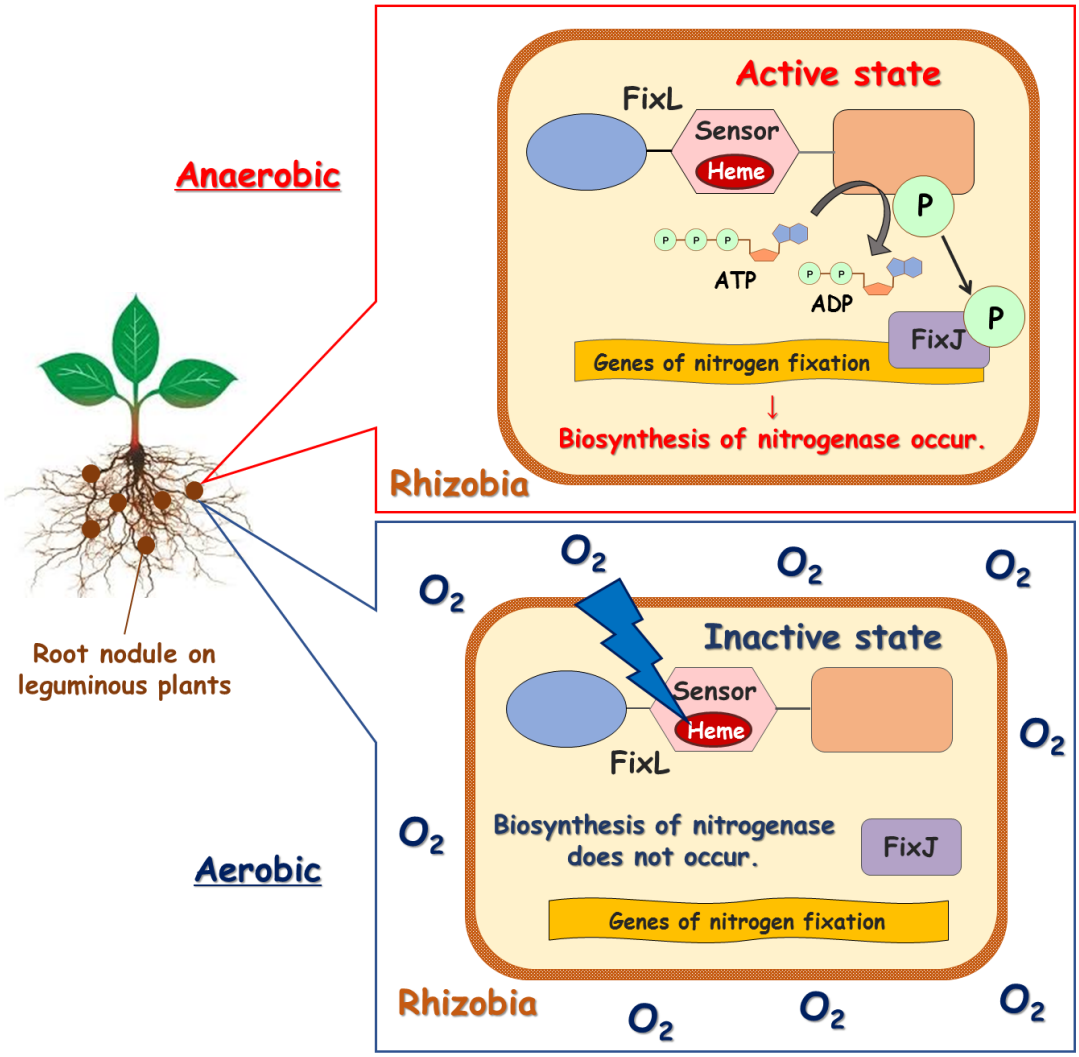

Figure 1. Mechanism of nitrogen fixation enzyme biosynthesis by the FixL/FixJ system with oxygen sensing system in nodule bacteria, Rhizobia. Molecular oxygen (O_2) is detected in rhizobia by

binding or dissociation of O_2 to the heme [5] in the sensor of the O_2 sensor protein, FixL. In the FixL that does not bind O_2, ATP [6] is hydrolyzed to remove one phosphate group. FixL transfer the phosphate group to FixJ. The FixJ binds to the gene coding nitrogen fixation enzymes, and the enzymes are biosynthesized (the upper balloon). On the other hand, when the O_2 is bonded to the heme in the FixL, these reactions do not proceed and nitrogen fixation enzymes are not biosynthesized (the lower balloon).

2. Research methods and results:

In this study, after evaluating the quality of purified full-lengths FixL and FixJ proteins with biochemical methods, structures were determined by small-angle X-ray scattering [7] and X-ray crystallography [8]. In the small angle X-ray scattering analysis, it is impossible to collect and analyze data if the shape and the oligomeric state of the sample particles in the solution are not homogeneous. Since the FixL protein is easy to aggregate, it was difficult to maintain a homogeneous state for a long time and the structural analysis by using the method was difficult at the beginning of the research. To overcome this problem, we established an equipment in the RIKEN beamline BL45XU at SPring-8. The equipment is assembled both with the column chromatography [9] for protein purification and the X-ray small-angle scattering measurement system, so that a small angle X-ray scattering measurement of a fresh protein sample that does not contain any aggregate, immediately after elution from a column has been enabled. Such a combined measurement system had already been introduced to the synchrotron radiation facilities in Western countries, but not in the Asia-Oceania countries. The research group measured the same samples in the synchrotron radiation facilities SOLEIL in France and proceeded the measurement and analysis by confirming the accuracy of the equipment introduced in SPring-8. Figure 2 is the first three-dimensional structure of FixL and FixL-FixJ complexes determined by the small angle X-ray scattering method and the X-ray crystal structure analysis based on the newly developed equipment in this study.

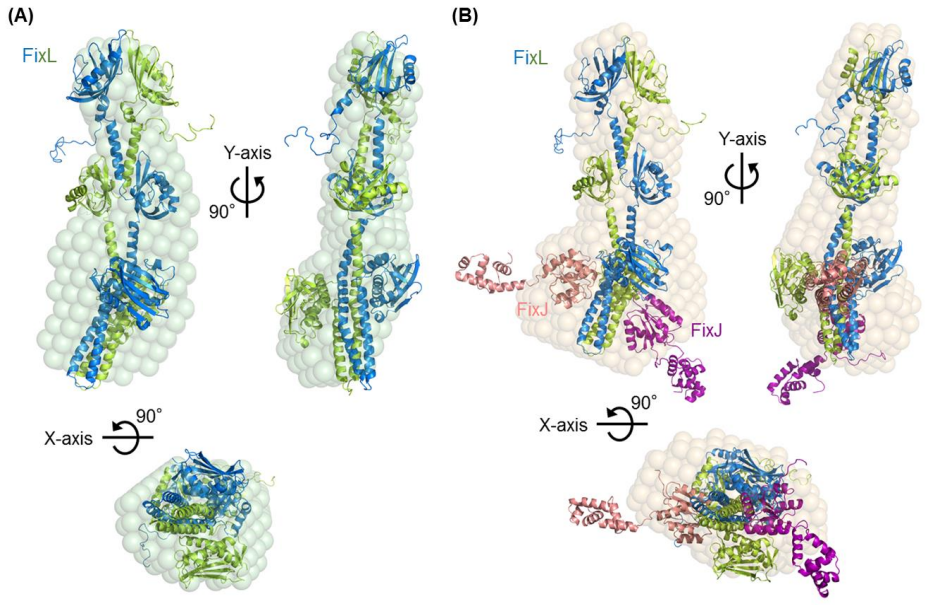

Figure 2. The structure of the full-length FixL (A) and its complex with the full-length FixJ (B)

revealed in this study. FixL forms a homodimer [10] as shown in the blue and green ribbon diagrams. The FixJ is shown in pink and magenta. One molecule of FixJ is bound to one molecule of FixL.

From these three-dimensional structures, it became clear that the FixL forms an intertwined homo-dimer, and its shape is like a short club. It was also found that there was no significant difference between the overall structures of oxygen-binding and -unbinding to heme of FixL. As a result, it was suggested that the information transmission caused by the detection of environmental factors such as oxygen is propagated by local small structural change. In this study, based on these structures, we proposed that the information is transmitted by the structural change, which occurs in the area between the sensor site and the phosphate group-binding site (Fig. 3). In the FixL-FixJ complex, it was found that only the phosphate-linked region of FixJ interacts with the FixL, and the rest of the area is flexible without interacting with the FixL. Because the transmission of phosphoric acid groups is a common mechanism for all two-component information transduction proteins, the interaction between the FixL-FixJ in this study is considered to be a common characteristic of the two-component information transduction system proteins. In addition, in the other proteins belonging to the two-component information transduction system, protein parts with various physiological functions are fused to the part corresponding to the flexible structure. Therefore, it can be thought that the two-component information transmission system protein became able to cope with various environmental factors by diversifying this flexible part in the process of evolution.

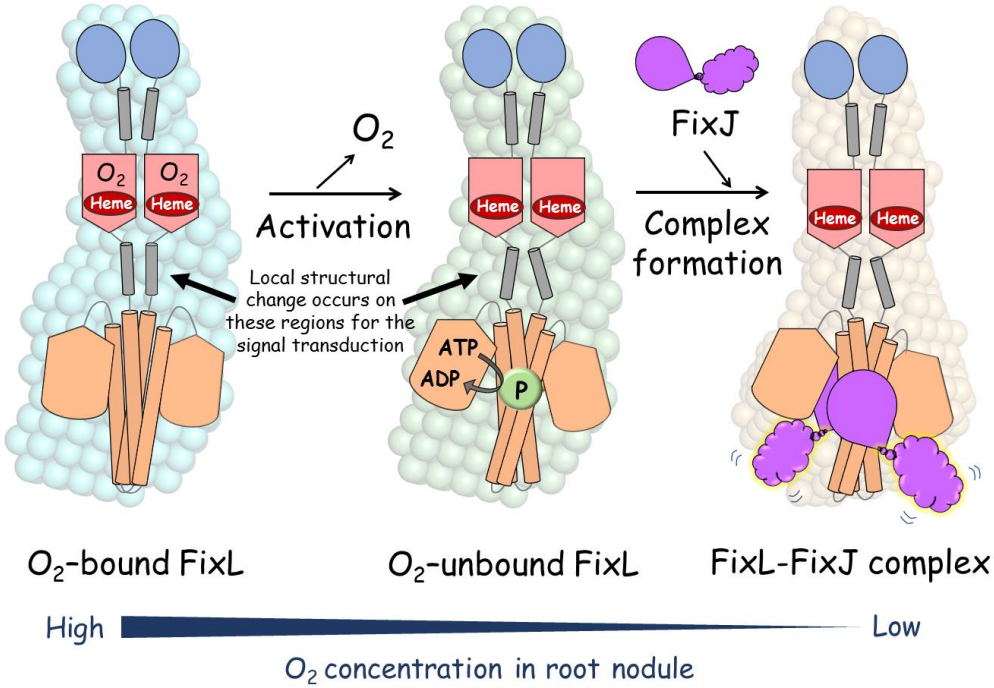

Fig. 3. A schematic diagram of the molecular mechanism of the oxygen sensing FixL/FixJ system, one of the two-component information transmission systems. When the oxygen concentration in the nodule bacteria is low, FixL and FixJ form a complex and it transmits the information by passing the phosphoric acid from FixL to FixJ.

3. Social significance and future prospects of this study:

The FixL/FixJ protein of soybean nodule bacteria, which is the target of this research, is indispensable for the supply of nitrogen nutrients essential to the growth of soybean, a host plant. Soybean is a highly nutritious and useful plant as reflected in its scientific name *Glycine max* (means “glycine, a kind of amino acid, is maximum”). In the current agriculture, the method of spraying the nodule bacteria solution in soybean plants is used, but there is the case where effect by this method cannot be expected depending on the environment. Based on the three-dimensional structure of the FixL/FixJ protein revealed in this study, the development of strains that can be effective even in such non-effective environment can be expected by improving soybean nodule bacteria. The two-component information transduction system, as the FixL/FixJ system, is an indispensable environmental response system for the living of microorganisms and higher plants. For example, the two-component communication system is used when pathogens infect the human body. On the other hand, this communication system has not been found at all in animals, including humans. By using the information of the elucidated three-dimensional structure, it is possible to develop a new type of antibacterial agent that does not affect animals by making a drug that acts on and specifically inhibits the two-component signal transduction system.

4. Glossary:

[1] Rhizobia and nitrogen fixation: Rhizobia are soil bacteria that form several millimeters in diameter size of root nodules in the roots of legumes. Rhizobia converts the nitrogen in the atmosphere to ammonia (nitrogen fixation) and supplies the ammonia as the readily available nitrogen nutrients to the host plant. The host plant has a symbiotic relationship with rhizobia by getting a photosynthetic product.

[2] "SPring-8" "SOLEIL": The world’s largest synchrotron radiation facility “SPring-8” located in Harima Science Park City in Hyogo Prefecture. This facility is managed by RIKEN and is capable of delivering what is now the strongest radiation in the world. SPring-8 stands for Super Photon ring-8 GeV (= 80 billion electron volts). “SOLEIL” (means "Sun" in French) is a relatively new synchrotron radiation facility run by the National Scientific Research Center CNRS and the nuclear agency CEA located near Paris in France. Synchrotron radiation is a powerful electromagnetic wave that occurs when electrons are accelerated to almost the same speed as the light, and the direction is bent by an electromagnet. In these facilities, a wide range of research has been conducted, from synchrotron radiation to nanotechnology, biotechnology, and industrial use.

[3] Nitrogenase: Enzyme in bacteria that perform nitrogen fixation. It catalyzes the reaction to convert nitrogen in the atmosphere to ammonia. A metal cluster is made of iron and molybdenum, resulting in deactivation when oxygen exists.

[4] Two-component information transduction system: An environmental response system for microorganisms and higher plants to perceive changes in the external environment and to adapt and survive these changes. It is called "two-component" information transmission system because it consists of two kinds of proteins "sensor histidine kinase" and "response regulator". When the sensor histidine kinase senses the environmental factor, the information is transmitted to the corresponding response

regulator via a phosphate group of ATP. The response regulator is required to adapt to the environment, fermentation gene expression and is involved in the control of the prime function. This function is achieved by the transfer of phosphoric acid group. FixL is corresponding to sensor histidine kinase and FixJ to the response regulator.

[5] Heme: A compound containing an iron atom in the center of the planar cyclic ring called porphyrin. Porphyrins are classified into several groups depending on the type and place of ring modification. Hemoproteins are defined as proteins that are functional only after incorporating a heme molecule, and in general, they appear red. Representatives of hemoproteins are hemoglobin, which delivers oxygen, cytochromes in the electron transport system, and peroxidases, which functions as an enzyme. The oxygen sensor protein FixL on this study is also hemoprotein, and the heme is used as an oxygen sensor.

[6] ATP (adenosine triphosphate): A compound in which three phosphoric acid groups are bonded to adenosine, the energy obtained by hydrolysis is used for life activities. Adenosine diphosphate (ADP) is the compound after releasing one phosphoric acid group by hydrolysis of ATP. Because many organisms use ATP as an energy source, they are sometimes called "the currency of biological energy."

[7] Small angle X-ray scattering method: A method of determining the structure of a target substance by irradiating X-rays which are a kind of short wavelength light to a substance in a solution, and the scattered X-ray scattering angle of scattered X-rays is analyzed in detail. This technique is generally used for the analysis of the internal structural of fine particles, liquid crystals, and alloys. Recently, this method is used to analyze the shape in the solution for biomolecules such as proteins.

[8] X-ray crystal structure analysis: A technique used to determine the structures of molecules. This is achieved by using a beam of short-wavelength X-rays to strike a crystal with an orderly arrangement of atoms and carefully analyzing the intensity of the scattered X-rays. The three-dimensional structures of a variety of biological molecules have been determined by this method.

[9] Column chromatography: Tubular container (column) filled with synthetic resins and porous gels. By shedding a solvent containing the object, it is possible to separate the object by differences in the size and the affinity of the molecules in the filler. In the purification of proteins, gel filtration column chromatography that separate the protein by the molecular size and ion exchange column chromatography that separates the protein by the difference in protein-specific isoelectric point are often used. In this study, we built and used the system, which fused the gel filtration column chromatography and the small angle X-ray scattering measurement.

[10] Dimer: An oligomeric state in which two molecules are assembled by physical and chemical action. The dimer of the same molecule is called homo dimer, the dimer by heterologous molecules is called the hetero dimer. The oligomeric state of FixL in this study is a homo-dimer.

5. Information of the journal publication:

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