

## Endocytosis and Endosymbiosis

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**Abstract** Symbioses are widespread in nature and occur between organisms that belong to a large variety of taxonomic divisions (Hentschel et al. 2000). Most often, only two partners are involved and the outcome may be either beneficial to both, i.e. mutualism, or detrimental to one of them, i.e. parasitism. Mutualism varies from simple protection against a hostile environment to an intimate cohabitation with exchange of essential nutrients. Important and well-studied examples are the symbiosis between nitrogen-fixing bacteria and plants of the Leguminosae family (approximately 750 genera and 20 000 species) and the arbuscular mycorrhizal interactions that involve more than 80% of land plants with fungi of the Glomeromycota. In the first case, plants profit through the supply of a nitrogen source, and in the second, through an uptake of phosphate. The microsymbionts benefit through the acquisition of carbon sources in a specific and exclusive ecological niche. In both types of interactions, the microsymbionts invade the plant host and the nutrient exchange takes place inside specialised plant cells. The establishment of the symbiosis is a complex process that requires the coordinated action of both symbionts and most probably the involvement of endocytosis in a number of critical events. In this chapter, we will describe both types of endosymbiosis in view of endocytosis and endocytosis-like processes.

### 1

#### Legume–Rhizobia Interactions

Even though molecular nitrogen makes up 78% of the atmosphere, the availability of fixed nitrogen is often a limiting factor for plant growth. Most organisms rely on the capacity of a restricted number of prokaryotes to reduce atmospheric nitrogen to ammonia that can be assimilated into organic molecules. The nitrogenase enzyme complex catalyses the process of biological nitrogen fixation, which accounts for approximately half of all the fixed nitrogen annually, the other half being provided by industrial production of ammonium. The rhizobium–legume symbiosis contributes up to 30% of the total biological nitrogen fixation and provides fixed nitrogen directly to a number of important agricultural crops (Vitousek et al. 1997).

Because nitrogenase is irreversibly inactivated by oxygen, biological nitrogen fixation requires anoxic or microaerobic conditions. During legume symbiosis, these conditions are created in newly formed root organs, the nodules, in which the bacteria reside (Schultze and Kondorosi 1998). Legume-nodulating bacteria are referred to as rhizobia and include taxonomically diverse genera, such as *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Sinorhizobium*, and the recently described *Methylobacterium*, *Burkholderia*, and *Ralstonia* sp. All these bacteria have in common the capacity to produce nodulation (Nod) factors, which are signalling molecules that play an essential role in initiation of nodule development and bacterial invasion (Oldroyd and Downie 2004). Nod factors are lipochitooligosaccharides and consist of an oligomeric backbone of  $\beta$ -1,4-linked *N*-acetyl-D-glucosaminyl residues, N-acylated at the nonreducing end, and decorated with species-/strain-specific substituents (D'Haese and Holsters 2002). Bacterial *nod*, *nol*, and *noe* genes code for the enzymes of the Nod factor biosynthesis and secretion pathway (Batut et al. 2004). Individual rhizobium strains may synthesise Nod factors of two to approximately 60 different types and host specificity is determined, at least in part, by qualitative and quantitative aspects of the Nod factor population. Also bacterial surface polysaccharides, such as exopolysaccharides (EPS), lipopolysaccharides (LPS), and succinoglycan are important in determining the host range and the host specificity of interactions (Frayse et al. 2003).

In general, two types of nodules are distinguished, determinate and indeterminate (Hirsch and LaRue 1997). Indeterminate nodules are elongated and have a persistent meristem that continually gives rise to new nodule cells. Representative legumes with indeterminate nodules are *Medicago sativa* (alfalfa), *M. truncatula*, *Pisum sativum* (pea), *Vicia* sp. (vetch), and *Trifolium* sp. (clover). Determinate nodules are round and lack a persistent meristem; they occur on *Lotus japonicus* and on tropical plants, such as *Glycine max* (soybean) and *Vicia faba* (broad bean). In both types, nodules are formed through a number of sequential developmental stages. We will describe the infection process of the indeterminate nodules, which are presumably the ancestral type (Sprent 2002) and include many processes that also take place during determinate nodule development.

Before their actual physical contact, the plant and the bacteria communicate through the exchange of signal molecules. Roots of host plants secrete flavonoids and betaines, which are sensed by the bacteria and induce the *nod* genes (Dénarié et al. 1996). The synthesis and export of Nod factors, in turn, trigger early nodulation responses in the host, including root hair membrane depolarisation, intracellular calcium oscillations, root hair deformation, and the initiation of cell divisions in the root cortex (Downie and Walker 1999; Miklashevichs et al. 2001). Rhizobial attachment to young growing root hairs is mediated by host lectins and bacterial adhesins (Kijne 1992).