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DISSERTATION SUMMARY

The role and regulation of *cycHJKL* operon in *Sinorhizobium meliloti*

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Rhizobium-species are soil bacteria, able to form symbiosis with the leguminous plants. On the roots of the host plant, some symbiotic nodules will appear, where *Rhizobium* bacteroids will fix the atmospheric dinitrogen into ammonia. In our laboratory we are doing research work on *Sinorhizobium meliloti*, the symbiotic partner of alfalfa *(Medicago sativa).*

During my work I was studying the role and regulation of *cycHJKL* operon from *Sinorhizobium meliloti.* The role of the proteins coded by this operon is the covalent binding of haem prostethic group to the c-type cytochrome apoprotein in the bacterial periplasm. After their biogenesis, the functional c-type cytochromes will remain in the periplasmic space, or will be builded in the membranes, having important role in different electron transport chains. The existence of a symbiotic electron transport chain, which is able to function during the microaerobic conditions of the symbiotic nodules, can produce the necessary ATP level for the energetically expensive nitrogen fixation, was published in 1993. The symbiosis-specific *cbb3*-type terminal oxidase complex contains c-type cytochromes and coded by the *fixNOQP* operon. During our experiments we demonstrated that the expression of our *cyc* operon is also induced microaerobically, like expression of *fixNOQP*, so it has an important role in the symbiosis. We showed that in our case sensing of the low oxygen level is not the responsibility of the well-known fixL/J two-component signal transduction system. We did not find any kind of oxygen sensor, which may have a role in the microaerobic induction of *cyc* operon. Searching the expression level of *fixNOQP* operon in *cyc* mutant bacteria, we find that this level can be higher or lower than in the wild type strain depending on the oxygen level present in the culture. By testing with triphenyltetrazolium chloride, we saw that under microaerobiosis the reduction capacity of *cyc* mutants is lower than in the wild type strain, so we hypothesized that the expression of *fixNOQP* operon under microaerobic conditions is regulated not only by FixL/J, but there is another regulatory system which senses the redox state of the cell.

The CycHJKL proteins are located in the membranes between the bacterial cytoplasm and the periplasmic space. Many laboratories are doing research on their exact role in the biogenesis of c-type cytochromes. CycH protein between its two transmembrane domains has a cytoplasmic loop and a long C terminal domain in the periplasmic space. Previous experiments show that CycH has a role in keeping the c-type cytochrome apoprotein until the covalent binding of the haem group established. In other species it was proved that the C terminal periplasmic part of CycH is functionally different from the other pars of the protein. In our experiments we saw that if there is an *in frame* mutation in one of the *cyc* genes, the biosynthesis of haem in the cytoplasm will be disturbed, and protoporphirin IX, the precursor of haem, is accumulated. If we made a mutation in the C terminal periplasmic part of CycH, there will be no changings in the level of PPIX. We have concluded that CycJ, CycK, CycL, proteins and the N terminal region of CycH, until the PP2982 mutation are nescessary for the normal biosynthesis of haem.

We found three domains on the C terminal of CycH: a tetratricopeptide (TPR), a hydrophobic helical stretch, and a methyl-coenzyme M reductase (MCR) domain. The role of these domains is known in other proteins, and with different complementation experiments we are studying their role in the biogenesis of c-type cytochromes.

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