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DISSERTATION SUMMARY

Maturation of hydrogenase enzymes in *Thiocapsa roseopersicina*

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T. roseopersicina is a purple photosynthetic sulfur bacterium, which harbours at least two membrane-bound (HynSL and HupSL) and one soluble (HoxEFUYH) [NiFe] hydrogenases (Rákhely et al. 2004). The maturation of these enzymes requires several accessory proteins, which are involved, among others, in metal incorporation, formation of the active centre, the proteolytic cleavage of the large subunit. In *T. roseopersicina* only few accessory genes could be found downstream from the *hupSL* genes. Using transposon-based random mutagenesis six independent hydrogenase minus mutants were isolated, and Southern analysis of these mutants indicated that they are scattered along the genome (Fodor et al. 2001).

Molecular analysis of three different mutant strains led to the identification of the Orf-s showing similarity to the [HupK (HoxV), HypC1, HypD, HypE], [HypC2, HynD] proteins of various bacteria. The genes are found in two distinct locuses, and transposon inserted into the *hypD*, *hypE* and *hynD* genes in the various mutants. The detailed analysis was executed to establish the role and the specificity of the *hupK*, $hypC_1$, $hypC_2$ and *hynD* genes (Maróti et al. 2003).

The localization of *hupK* gene is unusual: it is adjacent to the $hypC_1$, it occured generally with the hup genes. In frame deletion of *hupK* resulted around 90% loss of the membrane-bound activities (both Hup and Hyn), although the activity of the soluble hydrogenase was almost unaltered. RT-PCR experiments showed that the *hupK* and the $hypC_1$ were on the same transcript, although alternative transcripts and promoters could not be excluded.

The presence of at least two *hypC* genes in the genome was also surprising. None of the *HypC* proteins seemed to be strongly specific for any [NiFe] hydrogenases. The activity of each enzyme was affected by the mutation of both *hypC* genes.

In one hydrogenase mutant strain, the transposon inserted into the *hynD* gene resulting the loss of thermostable but not the heat labile hydrogenase activities. Carefully performed activity measurements on various mutants proved that the HynD protein being similar to the hydrogenase specific endoproteases was responsible for the maturation of the HynSL enzyme.

Homologous complementations with the appropriate genes proved the responsibility of these genes for the observed affects.

An expression vector-system was created in order to examine the maturation process of the hydrogenases in our host organism (Fodor et al. 2004). These vectors are useful for protein purification and for studying protein-protein interactions in a range of bacterial species.

References

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