

Abstract

Bacteria of the genera *Rhodococcus* and *Corynebacterium* are biotechnologically important microorganisms. This dissertation thesis focuses on the role of sigma (σ) factors of RNA polymerase in the regulation of gene expression in these bacteria. The *C. glutamicum* genome harbors 7 genes encoding σ factors, namely σ^A , σ^B , σ^C , σ^D , σ^E , σ^H and σ^M . With the exception of σ^M , these factors and their functions have been well described. The *R. erythropolis* genome encodes 21 putative genes for σ factors, but none of them has been analyzed in detail. The same holds for another biotechnologically useful bacterium, *R. opacus*. A group of genes whose expression is mediated by a single σ factor is called σ regulon. Overlaps of σ regulons are mainly caused by overlaps of recognition specificity of σ factors, i.e. when two or more σ factors recognize the same promoter. This thesis is focused on σ factors of group 4 (ECF) and their regulons: σ^D , σ^E , σ^H (*C. glutamicum*, *R. erythropolis*, *R. opacus*) and σ^M (*C. glutamicum*).

We used RNA-sequencing, *in vivo*, *in vitro* and *in silico* methods (homology modeling and molecular dynamics simulations) to achieve the intended aims. The overlap of σ^D and σ^H regulons was demonstrated in the natural hybrid promoter *Pcg0441*. Using the combination of *in vivo* and *in silico* methods, we were able to find interactions with σ^D responsible for the recognition of -35 region of *Pcg0441*. We created mutant variants of σ^M/σ^H -dependent promoter *Pcg0668* by site-directed mutagenesis of -35 (GGGAACC) and -10 (GTCAAA) region. We found the cause of the recognition specificity overlap of σ^M/σ^H factors for the *Pcg0668* promoter (Y¹⁰¹ of the σ^H -G₋₂₁ promoter, robustness of the σ^H factor near the RNA polymerase active site). Using *in vivo* and *in vitro* methods we managed to determine the consensus sequences of σ^D -dependent promoters (GTAAC for -35 region and GAT for -10 region), σ^E - and σ^H -dependent promoters (GGAA^{A/T} for -35 region and GTT for -10 region) of *R. erythropolis* and *R. opacus*. The functions of σ^D (cell wall integrity, response to the presence of tellurium compounds) and σ^H/σ^E (oxidative stress response, response to the presence of tellurium compounds) were characterized. An overlap of σ^H/σ^E -regulons of *R. erythropolis* was demonstrated and described. The highly integrated regulatory network controlled by σ factors has been described in *C. glutamicum* and partially also in *R. erythropolis* and *R. opacus*.

Key words: *Rhodococcus erythropolis*, *Corynebacterium glutamicum*, sigma factor, promoter, regulon, RNA polymerase