P048 Transcriptional regulation of nitric oxide reduction in *Ralstonia eutropha*

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Nitric oxide (NO) reduction in *Ralstonia eutropha* H16 is catalysed by the quinol-dependent NO reductase NorB. *norB* and the adjacent *norA* form an operon that is controlled by the sigma 54-dependent transcriptional activator NorR in response to NO. The N-terminal signalling domain of NorR contains a GAF motif and is supposed to interact with an effector. However, the molecular basis of the sensing mechanism is unknown. A NorR derivative lacking the signalling domain (NorR') activates the *norAB* promoter constitutively. Amino-acid exchanges within the GAF motif uncovered a Cys residue that is essential for promoter activation by NorR. The *norA* gene product negatively affects activation of the *norAB* promoter. NorA is a redox-active protein that contains one iron atom per molecule. The recognition sequence of NorR on the target DNA was investigated by DNaseI-footprint and gel-retardation assays using a MalE-NorR' fusion protein. Three putative NorR binding boxes GGT-(N_7)-ACC were identified upstream of *norA*. Mutations altering the spacing or the base sequence of these boxes resulted in an 80 to 90% decrease of transcriptional activation by wild-type NorR. The GT-(N_7)-AC core sequence is found in several proteobacteria upstream of genes encoding proteins of NO metabolism, including NO reductase (NorB), flavorubredoxin (NorV), NO dioxygenase (Hmp), and hybrid-cluster protein (Hcp).