

Abstract

σ^S is a sigma factor of RNA Polymerase activating the expression of the general stress response genes in *Escherichia coli*. Genes belonging to the σ^S network were identified over many years, but the total number of σ^S dependent genes and the details of their regulation remained still widely unknown. The goal of this work was the identification of all σ^S dependent genes by global transcription analysis and the determination of regulatory subgroups within the σ^S network. On the bases of the results of the global transcription analysis, σ^S dependent genes with a regulatory function should be identified and the genes controlled by these factors and the details of their regulation further examined. A σ^S dependent regulator together with the σ^S dependent genes under its control were defined as a 'regulatory module'. For a better understanding of the very complex regulatory relations within the σ^S network the concept of regulatory modules is an approach dissecting the many genes of the general stress response into clear subgroups which could be easier analyzed.

In this work the genes belonging to the σ^S -Regulon in *E. coli* were identified by global transcription analysis (microarray analysis). The wild type strain MC4100 was compared with its isogenic *rpoS* Mutant under three different growth conditions (transition into stationary phase, osmotic upshift, acid stress) triggering σ^S activity. Altogether 481 genes showed σ^S dependence under at least one of these conditions. Among these, 140 genes displayed σ^S -dependence under all three conditions tested and therefore were designated as the 'core genes' of the general stress response. Within the promoter regions of the core genes, the previously described DNA consensus motif of the -10 recognition site of σ^S was identified. On the basis of this data the consensus motif was defined with higher accuracy.

Finding the core genes *ydaM* and *yciR*, for the first time GGDEF/EAL proteins and the bacterial secondary messenger cyclic di-guanosine monophosphate (c-di-GMP) were identified as components of the σ^S network. YdaM and YciR antagonistically control the expression of curli fimbriae where YdaM acts positively and YciR negatively on the transcription of *csgD*, the central regulator of curli expression. *In vitro* analysis confirmed YdaM as a Diguanylatecyclase (synthesizes c-di-GMP) and YciR as a Phosphodiesterase (degrades c-di-GMP). Microarray analysis defined YdaM and YciR as specific regulators of curli expression instead of global regulators. For MlrA, another σ^S -dependent activator of curli expression, a binding site within the DNA upstream region of *csgD* was narrowed down to a range of approximately 40 basepairs. Overall the regulators YdaM, YciR, MlrA and CsgD together with the curli structural genes form a regulatory module within the σ^S network.

The genes of the acid stress response (*gadA*, *gadBC*, *hdeAB*, *hdeD*, *slp*, *ybaS*, *ybaT*, *yhiM*) and others, together with their regulators GadX and GadE were identified as another regulatory module within the σ^S network. It was shown that *gadE* (the central regulator of the glutamic acid dependent acid resistance in *E. coli*) is a σ^S and GadX dependent gene. Additional σ^S , GadX and GadE dependent genes, not considered as part of the acid stress response until now, were assigned to the acid stress module within the σ^S network. The genes of the acid stress module were categorized as distinct regulatory subgroups, according to differential dependence on GadX and GadE. One regulatory group consists of genes controlled by GadX solely, two other groups of genes are controlled by GadX and GadE in differing extent.

The present work took stock of all σ^S dependent genes. By defining two regulatory modules (curli and acid stress module) an approach to understand the regulation within the σ^S network was exemplarily demonstrated. The multitude of newly described σ^S dependent genes, particularly within the set of 140 core genes, provide a base for further research into the general stress response network. For the first time GGDEF/EAL proteins and the bacterial secondary messenger c-di-GMP were associated with the σ^S network. Therefore the present work is also a starting point for further investigations on the role of c-di-GMP in *E. coli*.