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NITROGEN REGULATION OF THE GLUTAMYL-tRNA SYNTHETASE GENE FROM THE CYANOBACTERIUM *SYNECHOCOCCUS SP.* PCC 7942.

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Although it has been long assumed that every organism contains 20 aminoacyl-tRNA synthetases, one for each amino acid, some organisms including cyanobacteria lack a glutaminyl-tRNA synthetase. In these cases the glutamyl-tRNA synthetase (GltX) plays a dual role charging both the tRNAglu and the tRNAgln with glutamic acid. The misacylated glu-tRNAgln is thereafter transformed in an amidation reaction into gln-tRNAgln (2, 3, 4). Given the central role of glutamic acid in nitrogen assimilation, as a product of the GS-GOGAT cycle, we have investigated the effect of the cellular nitrogen status on the operation of the glutamyl-tRNA synthetase from the unicellular cyanobacterium Synechococcus sp. PCC 7942. We found that the expression of gltX depends on nitrogen availability and is controlled by the global nitrogen regulator NtcA (1). gltX expression is low under nitrogen starvation and is activated, with the participation of NtcA, under nitrogen replete conditions (presence of ammonium or nitrate). The transcription start point (tsp) of this gene has been determined and three putative NtcA-binding sites centred at ^103.5, ^40.5 and -7.5 with respect to the tsp have been mapped. In vitro measurements of the affinity constants have demonstrated that NtcA exhibits different affinities for these sites, being the ^7.5 site the one with the lowest affinity. Our results are consistent with a model in which the level of expression of gltX is controlled by selective occupancy of NtcA-binding sites according to the cellular concentration the activator. Thus, low NtcA concentrations allow occupancy of the sites located at ^103.5 and ^40.5 that operate as activator sites, while high NtcA concentrations present under nitrogen starvation conditions, also permit occupancy of the site located at ^7.5, that functions as a repressor site, determining downregulation of gltX. In agreement with that model, a Synechococcus-derivative strain overexpressing NtcA that showed permanent downregulation of gltX likely resulting from high occupancy of the repressor site by NtcA.

In summary, the gltX promoter represents a novel class of NtcA-regulated promoter exhibiting a peculiar pattern of nitrogen-dependent expression. The presence of activator and repressor sites in this promoter allows a fine tuning of its expression to nitrogen availability relying upon the cellular concentration of NtcA.

Key references

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