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THE *HETZ* GENE REGULATES HETEROCYST FORMATION IN *ANABAENA* SP. STRAIN PCC 7120

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ABSTRACT

To form a complex multicellular organism, stem cells must differentiate into each cell/tissue type along proper spatiotemporal scales. The study of differentiation and organismal development has historically been conducted in prokaryotes due to their genetic and morphological simplicity. *Anabaena* sp. strain PCC 7120 is a multicellular filamentous cyanobacterium that differentiates a morphologically distinct secondary cell type, the heterocyst, in response to a lack of combined environmental nitrogen. Heterocysts are regularly spaced along filaments and fix atmospheric dinitrogen to maintain organismal viability in its absence. Previous work suggested that the *hetZ* gene is involved in heterocyst differentiation, but the insertional mutants created produced inconsistent phenotypes, so a specific role was not assigned. In this work, a clean *hetZ* mutant incapable of heterocyst differentiation was generated and the mutation was complemented with the reintroduction of *hetZ* alone. Overexpression of *hetZ* bypassed a mutation of *hetR*, the master regulator of heterocyst differentiation that controls biological pattern formation, but not a mutation of *hetP*, a regulator of commitment to a differentiated cell fate, which places *hetZ* roughly between these processes. A protein-protein interaction study showed that HetZ interacts with both HetR and itself. Assessment of transcriptional fusions between the *hetZ*, *hetR*, *hetP*, and *patS* (an inhibitor of HetR) promoter regions and GFP, and overexpression of HetR in a *hetZ* mutant resulted in the differentiation of heterocyst-like cells, together indicated that HetZ may act in concert with HetR as an early regulator of development. Taken together, these data describe a non-linear pathway of regulation leading to heterocyst development governed by both HetR and HetZ.