

## Ancient association of cyanobacterial multicellularity with the regulator HetR and an RGSGR pentapeptide-containing protein (PatX)

### Summary

One simple model to explain biological pattern postulates the existence of a stationary regulator of differentiation that positively affects its own expression, coupled with a diffusible suppressor of differentiation that inhibits the regulator's expression. The first has been identified in the filamentous, heterocyst-forming cyanobacterium, *Anabaena* PCC 7120 as the transcriptional regulator, HetR, and the second as the small protein, PatS, which contains a critical RGSGR motif that binds to HetR. HetR is present in almost all filamentous cyanobacteria, but only a subset of heterocyst-forming strains carry proteins similar to PatS. We identified a third protein, PatX, that also carries the RGSGR motif and is coextensive with HetR. Amino acid sequences of PatX contain two conserved regions: the RGSGR motif and a hydrophobic N-terminus. Within 69 nt upstream from all instances of the gene is a DIF1 motif correlated in *Anabaena* with promoter induction in developing heterocysts, preceded in heterocyst-forming strains by an apparent NtcA-binding site, associated with regulation by nitrogen-status. Consistent with a role in the simple model, PatX is expressed dependent on HetR and acts to inhibit differentiation. The acquisition of the PatX/HetR pair preceded the appearance of both PatS and heterocysts, dating back to the beginnings of multicellularity.