Heterocyst formation under the control of a cell-specific antisense RNA

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Commentary: A heterocyst antisense RNA controlling its sense mRNA target to develop a cell-specific regulation

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Cyanobacteria, prokaryotes doing oxygenic photosynthesis, are important fixers of CO_2 in oceans and lakes. While most of cyanobacteria assimilate only combined nitrogen compounds (such as nitrate, ammonium, urea), certain strains are able to fix atmospheric nitrogen when other sources of N are lacking. However, the enzyme responsible for N_2 fixation is inactivated by oxygen and then certain filamentous strains develop specialized cells, called heterocyst, in which there is no production of O_2 via photosynthesis (reviews about heterocysts: (Flores and Herrero, 2010, Herrero et al., 2016, Magnuson, 2019, Meeks et al., 2002). Also key enzymes involved in the photosynthetic fixation of CO_2 like Rubisco and phosphoribulokinase are absent whereas others are largely diminished. Furthermore, the heterocysts envelope is different and decreases the entry of gases inside these cells together with activation of terminal oxidases to cope with any residual oxygen interfering with its nitrogen-fixing function. When vegetative cells evolve into heterocysts, the differentiation process presents multiple structural and functional changes in these cells that require an extensive modification of gene expression under the control of the global nitrogen regulator NtcA and HetR, an specific regulator of its differentiation.

Non-coding RNAs (sRNAs) have been sown to regulate complex transcriptional networks in all life kingdoms. Although the majority of non-coding RNA is present in eukaryotes, many non-coding RNAs have been shown to regulate virtually any adaptive response of bacteria to environmental changes (Jimenez-Zurdo and Robledo, 2017). The canonical activity of prokaryotic non-coding RNAs involves base pairing to coding mRNAs through which their stability or translation is regulated and, in many cases, involves extensive perfect complementarity as it is the case for cis-acting antisense sRNAs (asRNAs). Natural antisense RNAs (asRNA) are a diverse group of bacterial RNAs first discovered many decades ago however their biological relevance has been appreciated more recently. Antisense transcript regulatory mechanisms affect different levels of gene expression including: transcription interference, transcription attenuation, translation stimulation or inhibition, and RNA stability (Saberi et al., 2016). Several advantages can be referred to asRNA-based regulation such as shorter regulatory times (direct targeting of the sense mRNA), specificity

of gene regulation based on base pairing (including potentially simultaneous trans effects at different homologous loci) and the capacity of asRNA to modulate conformation interactions depending on temperature and other environmental changes. Abundant nitrogen-regulated antisense transcripts have been detected in cyanobacteria strains including the nitrogen fixing *Nostoc* PCC7120 (Flaherty et al., 2011, Mitschke et al., 2011). Only few (6) of them have been characterized: IsiR, regulates the degradation of the *isiA* mRNA; As1_flv4, down-regulates the *flv2-4* operon; PsbA2 and 3, upregulate the expression of *psbA2* and *psbA3* by stabilizing their coding mRNAs; RblR, a positive regulator of the RubisCO; pilR, a negative regulator of pilA11 and finally furA which modulates the concentration of FurA, a regulator of iron assimilation is the only characterized in *Nostoc* PCC 7120.

In this issue, Olmedo-Verd et al. characterized in *Nostoc* PCC 7120, a first heterocyst-specific asRNA, as_glpX, antisense to the glpX gene encoding the Calvin cycle enzyme sedoheptulose-1,7-biphosphatase/fructose-1,6-biphosphatase (SBPase) involved in CO_2 assimilation. First, the authors demonstrated its existence and transcriptional induction upon removal of combined nitrogen. This 458 nucleotide long asRNA requires the presence of HetR to be transcribed. By introducing a *gfp* gene under the control of the as_glpX promoter, the authors demonstrated that the asRNA is expressed only in heterocysts (and not in vegetative cells) since the very early stages of differentiation. In the as_glpX promoter a DIF1 motif was found, a cis-sequence presents also in other promoters of early differentiation genes (Muro-Pastor, 2014, Muro-Pastor et al., 2017).

By constructing a strain overexpressing the as_glpX in all cells and not only in heterocyts (only 10-15% of cells), the authors demonstrated its repressive effect on the glpX mRNA. Smaller RNAs were detected with the glpX5'-probe suggesting the triggering of mRNA degradation by the antisense transcript. Also, the concentration of the SBPase protein was lower in the overexpressing asRNA mutant confirming that as_glpX induces the degradation of glpX mRNA and repression of SBPase protein accumulation in heterocyst cells. This perturbation of antisense expression diminished the concentration of SBPase protein and largely affects culture growth and photosynthesis under high light conditions (400 $\mu E\ m^{-2}\ s^{-1}$). The cells grew slower than control cells and their photosynthesis saturated at lower light intensities and the maximal rate was about 50% of that of control cells.

In conclusion, as_glpX is a first heterocyst specific asRNA with a known function in early stages of heterocyst differentiation to control photosynthetic O_2 production and permit nitrogenase function. Other HetR and NtcA regulated asRNAs may also contribute to fine-

tune gene expression regulation during cyanobacterial differentiation. These results open wide perspectives for the research of regulatory mechanisms involved in bacterial differentiation.

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