The cyanobacterial homologue of the RNA chaperone Hfq is essential for motility of *Synechocystis* sp. PCC 6803

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The ssr3341 locus was previously suggested to encode an orthologue of the RNA chaperone Hfq in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Insertional inactivation of this gene resulted in a mutant that was not naturally transformable and exhibited a non-phototactic phenotype compared with the wild-type. The loss of motility was complemented by reintroduction of the wild-type gene, correlated with the re-establishment of type IV pili on the cell surface. Microarray analyses revealed a small set of genes with drastically reduced transcript levels in the knockout mutant compared with the wild-type cells. Among the most strongly affected genes, *slr1667, slr1668, slr2015, slr2016* and *slr2018* stood out, as they belong to two operons that had previously been shown to be involved in motility, controlled by the cAMP receptor protein SYCRP1. This suggests a link between cAMP signalling, motility and possibly the involvement of RNA-based regulation. This is believed to be the first report demonstrating a functional role of an Hfq orthologue in cyanobacteria, establishing a new factor in the control of motility.

**INTRODUCTION**

Cyanobacteria constitute a diverse group of photoautotrophic bacteria that perform oxygenic photosynthesis and are present in almost any environment on this planet, such as freshwater, oceans, the surface of rocks, desert soil or the polar regions. Analogous to enterobacteria, in which all responses to environmentally relevant stress conditions have been suggested to include at least one small regulatory RNA (sRNA) as part of the regulon (Gottesman, 2005), cyanobacteria are likely to possess sophisticated riboregulatory mechanisms. Up to now, there have only been a few studies that have identified or functionally characterized sRNAs in cyanobacteria. Essentially, these investigations have been focused on the unicellular marine Prochlorococcus group (Axmann et al., 2005; Steglich et al., 2008), the unicellular freshwater cyanobacterium *Synechocystis* sp. PCC 6803 (Dühring et al., 2006) and the filamentous strain *Anabaena* sp. PCC 7120 (Hernandez et al., 2005). In the latter two cases, the involvement of an antisense RNA in iron homeostasis was demonstrated. The *Synechocystis* sp. PCC 6803 antisense RNA IsrR proved to negatively control the expression of the accessory photosynthesis protein IsiA (iron-stress-induced protein A), which dramatically accumulates upon several environmental challenges including iron depletion (reviewed by Singh & Sherman, 2007).

In *Escherichia coli* and closely related bacteria, the functional efficiency and stability of a considerable number of sRNAs is dependent on, or decisively enhanced by, the RNA-binding protein Hfq (Sledjeski et al., 2001; Möller et al., 2002; Zhang et al., 2002; Geissmann & Touati, 2004; Gottesman, 2004; Udekwu et al., 2005; Kawamoto et al., 2006; Urban & Vogel, 2008; Pfeiffer et al., 2007; Večerek et al., 2007). The homohexameric Hfq protein is structurally and functionally similar to eukaryotic Sm proteins (Valentin-Hansen et al., 2004). Its regulatory activity for *E. coli* has been postulated to facilitate the coupled degradation of sRNA–mRNA-duplexes in concert with the major
nuclease, RNase E (Massé et al., 2003; Morita et al., 2005). Further molecular functions of Hfq include stability control of mRNAs by regulation of polyadenylation (Hajnsdorf & Regnier, 2000; Folichon et al., 2003, 2005; Mohanty et al., 2004) and sequestering RNase E cleavage sites (Folichon et al., 2003; Moll et al., 2003), thereby influencing poly(A)-dependent RNA turnover and endonucleolytic restriction, respectively. Hfq seems to contribute to overall translational performance by accelerating the activity of the tRNA nucleotidyltransferase (Scheibe et al., 2007) and it also binds tRNAs (Lee & Feig, 2008).

Genetic inactivation of hfq in diverse eubacteria caused pleiotropic physiological effects (Tsui et al., 1994; Muffler et al., 1996, 1997) and revealed a fundamental role of Hfq in the virulence of pathogenic bacteria (Robertson & Roop, 1999; Sonnleitner et al., 2003; Christiansen et al., 2004; Ding et al., 2004; Sharma & Payne, 2006; Sittka et al., 2007; Wilson et al., 2007; Luccetti-Miganeh et al., 2008). In this context, there are several reports on Hfq-mediated adaptation to changing environmental conditions, exemplified by Brucella abortus (Robertson & Roop, 1999; Gee et al., 2005; Valderas et al., 2005), Salmonella typhimurium (Sittka et al., 2007) and Azorhizobium caulinodans, as well as the phototrophs Rhodobacter capsulatus (Kaminski et al., 1994; Kaminski & Elmerich, 1998; Drepper et al., 2002) and Rhodobacter sphaeroides (Glaser et al., 2007). In contrast, no apparent phenotype emerged from an hfq knockout in Staphylococcus aureus (Bahn et al., 2007). Although not all eubacterial genomes encode an Hfq protein, an Hfq-like protein has recently been identified in Archaea (Nielsen et al., 2007). Importantly, although this Methanocaldococcus jannaschii protein differs from the well-characterized eubacterial Hfq proteins in length and amino acid sequence, it does restore complex phenotypes to an E. coli hfq deletion strain (Nielsen et al., 2007).

Initial attempts to explore microbial genomes for Hfq homologues failed with regard to cyanobacteria (Sun et al., 2002). However, broadening the criteria and specifically targeting proteins with a possible 5′ motif resulted in the identification of candidate genes in a variety of cyanobacteria (Valentin-Hansen et al., 2004; see Supplementary Fig. S1, available with the online version of this paper). Within the cyanobacterial genus Prochlorococcus, hfq has been lost in several of the sequenced strains (Axmann et al., 2005). Nevertheless, the naturally hfq-deficient Prochlorococcus MED4 expresses at least 21 different non-coding RNAs (Axmann et al., 2005; Steglich et al., 2008). Therefore, the functional importance of Hfq in cyanobacteria has remained entirely unclear and has not been experimentally addressed thus far.

Here we show that the hfq candidate gene in Synechocystis sp. PCC 6803 can be knocked out without any detrimental effects on cell growth. However, the most striking change, which could be completely reversed by reintroduction of the wild-type (WT) gene, is the loss of motility correlated with the absence of type IV ‘thick’ pili on the cell surface. Microarray analyses suggest a number of genes that depend on Hfq; among them, there are two operons that are controlled by the cAMP receptor protein (CRP) SYCRP1, an established regulator of cell motility, as well as several genes with unknown function and the chaperone genes groES and groEL.

**METHODS**

**Bacterial strains and growth conditions.** The motile strain of Synechocystis sp. PCC 6803 used in this study was originally obtained from S. Shestakov (Moscow State University, Russia) and propagated on BG11 agar plates (Rippka et al., 1979; 0.75%, w/v, agar). Liquid cultures of Synechocystis sp. PCC 6803 WT and mutant strains were grown at 28 °C in BG11 medium containing 10 mM MES buffer (pH 8.0) under continuous illumination with white light of 75 μmol photons m⁻² s⁻¹ and a continuous stream of air. For the mutants described below, antibiotics were used at the following concentrations: chloramphenicol (Cm), 7 μg ml⁻¹; kanamycin (Km), 80 μg ml⁻¹.

E. coli strain DH5α was used for all plasmid constructions; strain J53 was used for conjugation with Synechocystis sp. PCC 6803. E. coli cultures were generally grown in LB medium, supplemented with antibiotics at standard concentrations as appropriate.

**Plasmids and mutagenesis.** For insertion mutagenesis, the hfq ORF (locus ssr3341) (Kaneko et al., 1996) was amplified as a 492 bp fragment including 100 bp of the 5′- and 179 bp of the 3′-flanking regions (putative promoter and terminator regions, respectively) using primers P1 and P2 (see Supplementary Table S1, available with the online version of this paper) and ligated into the pGEM-T-Easy vector (Promega). Eventually, the chloramphenicol resistance cassette from pACYC184 (New England Biolabs) was inserted into an XmaI site within ssr3341 (Fig. 1) which had been converted to blunt ends using the Klenow fragment. This construct was used to transform Synechocystis as described by Ermakova et al. (1993). Transformants were restreaked six times and analysed by Southern blot hybridization and PCR to detect the level of segregation of WT genome copies.

Complementation of the Δhfq disruption was initially approached by amplification using primers P3 and P4 of an 1111 bp fragment, including ssr3341, flanked by 500 bp in the 5′- and 398 bp in the 3′-direction (including its own putative promoter and terminator regions). The amplicon was ligated into the pDrive vector (Qiagen), excised by PstI/Sall digestion and finally inserted into the conjugative, self-replicating vector PVZ231 (Zinchenco et al., 1999), replacing the plasmid’s Cm resistance cassette. The resulting plasmid, pVZ-Δhfq15, was transferred to Δhfq mutant cells by conjugation (Zinchenco et al., 1999), wherein exconjugants were selected on BG11 agar containing up to 80 μg Km ml⁻¹ and 7 μg Cm ml⁻¹; the latter one due to the insertion in the disruptant background. Mutants engineered with the equivalently constructed plasmid pVZ-hfqS2, which contained a single point mutation at position 121 in the hfq coding sequence which leads to an in-frame stop codon and thus to a truncated polypeptide (see Supplementary Fig. S1), served as a negative control for complementation experiments.

**Motility assays.** Phototactic movement was examined as described by Wilde et al. (2002) using BG11 0.5% (w/v) agar plates supplemented with 10 mM TES (pH 8.0) and 10 mM glucose. The plates were put into opaque boxes with an open slit (3 cm in width) and illuminated with white light of 1–3 μmol photons m⁻² s⁻¹ at 28 °C. Phototactic movement was documented after 2 weeks using a Plustek OpticPro ST148 Scanner.
**RNA isolation and Northern hybridization.** Exponentially growing *Synechocystis* sp. PCC 6803 liquid cultures (OD$_{750}$ 0.6–0.8, Ultron II, Pharmacia) were collected by quenching on ice and immediate centrifugation at 0–4 °C. Cell pellets were resuspended in 1 ml TRizol reagent (Invitrogen) per 20 ml aliquot and total RNA was isolated by following the manufacturer’s instructions. The purified RNA was quantified using a NanoDrop ND-1000 spectrophotometer (PepTide Biotechnology) separated by electrophoresis on 1.3 % agarose formaldehyde gels and blotted onto Roti-Nylon plus (Sambrook & Russell, 2001). Hybridization probes were generated either by random prime labelling (Rediprime II labelling kit, GE Healthcare) or by PCR products from the T7 promoter in the presence of dCTP (Hartmann Analytic) or by in vitro transcription of PCR fragments from the T7 promoter in the presence of [α-32P]UTP (Hartmann Analytic) using the T7 polymerase MaxiScribe kit (Ambion). Following prehybridization for at least 30 min in 50 % denaturated formamide, 7 % SDS, 250 mM NaCl and 120 mM sodium phosphate pH 7.2 at 68 °C for DNA probes and 68 °C using riboprobes, the labelled probes were added and hybridized for 3–16 h. The membranes were rinsed in 2× SSC/0.5 % SDS and washed in two subsequent 15 min steps at 68 °C in 2× SSC/0.5 % SDS and 0.1× SSC/0.1 % SDS, respectively. Signals were detected and analysed on a Personal Molecular Imager FX system with Quantity One software (Bio-Rad). All DNA oligonucleotides are listed in Supplementary Table S1.

**Transmission electron microscopy.** Samples of the different *Synechocystis* strains were collected from colonies on the surface of the agar plates by resuspending in BG11 medium. For electron microscopy, the cyanobacteria were negatively stained with 0.5 % aqueous uranyl acetate (Golecki, 1988). Micrographs were taken with a Philips CM10 electron microscope (Fei Company) operating at 80 kV, with integrated BioScan camera model 792 and Digital Micrograph software (Gatan).

**Microarray analysis.** Microarray experiments were carried out as two-colour hybridizations on custom-designed 8-pack 15K *Synechocystis* sp. microarrays (AMADID 016989) from Agilent Technologies. The RNA integrity and the amount of total RNA were measured with a Bioanalyzer 2100 (Agilent Technologies). Per sample, 3 μg total RNA was reverse transcribed with Cyber dye post-labelling kit (GE Healthcare). First-strand cDNA probe (300 ng) was labelled by the ‘post-labelling’ (aminol allyl) method with Cy3 and Cy5. Labelling efficiencies and cDNA amounts were determined with a Nanodrop ND-1000 spectrophotometer (Kisker). In order to compensate specific effects of the dyes and to ensure statistically relevant data analysis, a colour-swap dye-reversal was performed (Churchill, 2002). Labelled samples were mixed, hybridized to 15K custom-made microarrays and washed according to the supplier’s protocol (Agilent Technologies). Scanning of microarrays was performed with 5 μm resolution using a DNA microarray laser scanner (Agilent Technologies). Features were extracted with an image analysis tool version A.9.5.3 using the GE2-V5.95_Feb07 protocol (Agilent Technologies). Data analysis was carried out on the Rosetta Inpharmics platform Resolver Version 7.0. Ratio profiles were combined in an error-weighted fashion with Resolver to create ratio experiments. A twofold change expression cut-off for ratio experiments was applied together with anti-correlation of ratio profiles, rendering the microarray analysis set highly significant (P>0.05), robust and reproducible.

**RESULTS**

**Inactivation of ssr3341 in Synechocystis sp. PCC 6803 and growth characteristics of the deletion strain**

To investigate the function of a previously predicted cyanobacterial Hfq orthologue (Valentin-Hansen et al.,
Hfq and cyanobacterial motility

Motility analysis and cell surface images

Phototactic motility of the Δhfq knockout strain was assayed in comparison with the WT under unidirectional white light. WT cells moved towards the light source, whereas Δhfq mutant colonies showed no motility, as evaluated from the smooth edges of the spot (Fig. 2), within which the mutant cells were evenly distributed. Because loss of phototactic motility may arise from the absence of motility-mediating (thick) type IV pili and from hyperpiliation as well (Bhya et al., 2000), mutant and WT cells were subjected to electron microscope examinations. Whereas WT cells possess mainly two types of pili on their surface, thin pili and thick pili (Bhya et al., 2000; Yoshihara et al., 2001), piliation appeared to be completely absent from Δhfq mutant cells (Fig. 3b). In order to exclude possible secondary effects caused by the inserted resistance cassette or by unrelated mutations, we restored the WT phenotype (i.e. motility) by introducing a complementing hfq copy into the mutant cells. Since the absence of pili was also accompanied by a loss of natural competence for transformation (data not shown) we used the conjugative shuttle vector pVZ-Phfq15, harbouring the hfq gene flanked by its own promoter and terminator regions for complementation. Additionally, conjugation was performed with the negative control vector pVZ-hfqS2, containing the same DNA fragment with a single point mutation in the hfq gene leading to a truncated ORF (Supplementary Fig. S1). The motility analysis (Fig. 2) including the resulting strains hfq-K (complementation) and hfq-S (negative control) demonstrated that the introduction of an independent, extrachromosomal WT copy of hfq reverted the mutant back to the WT phenotype. Cells containing the original pVZ321 vector (Zinchenko et al., 1999) apparently slowed down the movement of the cells towards the light source. This effect is assumed to be a consequence of a shifted cellular energy balance caused by the additional replicon and the accompanied expression of foreign protein (Bentley et al., 1990). The hfq-S strain harbouring the truncated version of hfq did not move towards the light, indicating that the non-motile phenotype was exclusively due to the loss of Hfq. In accordance, the genetic complementation restored the piliation on the mutants’ cell surface, as is shown in Fig. 3(c).

Screening target genes for Hfq using DNA microarray analysis

To analyse the effects of the hfq disruption on gene expression, microarrays that cover 3264 protein-coding genes of the Synechocystis sp. PCC 6803 genome (Eisenhut et al., 2007) were hybridized with total RNA from two independent preparations of WT and mutant cells. Thirty-one genes displayed statistically significant changes in transcript levels in the Δhfq knockout background (Table 1). Intriguingly, these genes emerged as being almost exclusively repressed in the disruptant, while only two genes (sll3445 and sll1942) showed an inverse behaviour. Among the most strongly responding genes were slr1667, slr1668, slr2015, slr2016 and slr2018, which had previously been described as five of six target genes for SYCRP1, a cyanobacterial CRP, encoded by sll1371 (Yoshimura et al., 2002a, b). The microarray analysis revealed several additional genes whose expression was significantly repressed in the Δhfq mutant that are also located in tandem on the chromosome. Besides slr2075 and slr2076, encoding the chaperonins GroEL and GroES, respectively, expression of slr0150 and slr0151, the former encoding the ferredoxin PetF, appears to be co-regulated in an Hfq-dependent manner. In addition, transcript accumulation of the gene pairs slr1429–sll1430 and sll1239–sll1240 as well as of several single genes (Table 1) was reduced in the Δhfq mutant. However, apart from the two SYCRP1-regulated operons, no functions have yet been assigned to most of these putative Hfq targets.

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Fig. 2. Directional motility assay. Exponentially growing cells (OD750 ~0.4) were spotted onto a 0.5% agar BG11 plate without antibiotic supplements and exposed to an unidirectional light source (arrow) for 2 weeks. WT, wild-type; pVZ321, WT strain harbouring the unmodified pVZ321 plasmid; Δhfq, disruptant; Δhfq-K, complemented strain harbouring pVZ-Phfq15; Δhfq-S, negative control strain harbouring pVZ-hfqS2, containing an internal stop codon within the hfq ORF.
Verification of the microarray data by Northern blot analyses

The results of the microarray experiments were verified by Northern blot analyses using hybridization probes for the initial and terminal genes in operons slr1667–slr1668 and slr2015–slr2016–slr2017–slr2018. As ssr2848 (encoding a hypothetical protein) appeared to be the second most affected gene in the microarray analysis, its differential expression was also chosen to be verified, along with slr1764, which has been annotated as a CAMP-binding protein, TerE. Fig. 4 shows confirmation that transcript accumulation of representatives of both the above-mentioned operons is drastically reduced in Δhfq. In particular, slr1667 and slr1668 mRNAs were not detectable in the mutant by Northern blot hybridization, suggesting a complete repression of these genes owing to the absence of Hfq (Fig. 4a). The same conclusion can be drawn from the probing of ssr2848 and slr1764 (Fig. 4b), again supporting the validity of the dataset. Furthermore, the expression levels of slr2015 and slr2018, as detected by Northern blot analysis, corresponded with the microarray data: each gene showed a rather moderate repression (Fig. 4a).

The photosynthesis-related transcript psbA2 was hybridized as a control, demonstrating that the observed effects were not based on a global, rather non-specific shut-down of gene expression in the Δhfq disruptant (Fig. 4b).

Three further genes were probed that are known to be essentially involved in cellular motility (Kaimei et al., 2001; Yoshihara et al., 2001; Yoshihara & Ikeuchi, 2004; Panichkin et al., 2006). Unlike the microarray dataset, the Northern blot data revealed that pilA1, encoding the main structural component of type IV pili, showed a slight decrease in transcript abundance in Δhfq. In contrast, transcript accumulation of pilB1 and spkA, encoding a nucleoside triphosphatase (NTPase) and a serine/threonine kinase, respectively, was increased in the mutant (Fig. 4b, c). Further, Northern blot hybridizations of total RNA isolated from the complementation strain Δhfq-K and the negative control strain Δhfq-S, applying the slr1667 probe, demonstrated the restoration of the WT phenotype at the molecular level (Fig. 5). Thus, these data clearly show Hfq-dependent regulation of motility-related genes and operons as well as of several further genes, whose function remains to be elucidated.

DISCUSSION

The Synechocystis sp. PCC 6803 Hfq candidate protein is only 70 aa in length and its sequence is rather diverse compared with functionally characterized RNA chaperones. Yet the severe phenotypic effects and changes in mRNA abundances observed here resemble Δhfq mutants in other model organisms and therefore provide circumstantial evidence that ssh3341 does encode a cyanobacterial Hfq protein. Synechocystis sp. PCC 6803 is able to move towards light sources in a process called twitching motility (Bhaya, 2004) that might enhance the fitness of cyanobacterial cells in biofilms, especially with regard to diurnal changes in illumination (for reviews on motility with regard to biofilm formation and light adaptation see Bhaya, 2004; Nudelman & Kaiser, 2004). The central morphological prerequisite for this phototactic movement is the presence of type IV pili (McBride, 2001), which are also known to be essential for their natural competence (Yoshihara et al., 2001; Nakasugi et al., 2006). Both of these pili-dependent mechanisms were lost as a consequence of hfq inactivation in Synechocystis sp. PCC 6803, and electron microscopy provided evidence for the loss of type IV pili on the mutants cell surface. Targeting molecular details for the non-related Δhfq phenotype, microarray analysis revealed the two operons slr1667–slr1668 and slr2015–2018 to be most strongly repressed in the Δhfq mutant compared to WT cells. The decrease in transcript levels of slr1667–slr1668 in Δhfq is comparable to that previously observed in a disruption mutant of sycp1 (Yoshimura et al., 2002a; Panichkin et al., 2006). This gene...
Table 1. Differentially expressed genes in *Synechocystis* sp. PCC 6803 WT and Δhfq mutant as determined by microarray analysis

<table>
<thead>
<tr>
<th>Genomic locus</th>
<th>Description</th>
<th>Fold change*</th>
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<tbody>
<tr>
<td>slr1667</td>
<td>Target of SYCRP1</td>
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</tr>
<tr>
<td>slr1668</td>
<td>Target of SYCRP1</td>
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<td>srr2848</td>
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<td>sll2996</td>
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</tr>
<tr>
<td>slr2018</td>
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</tr>
<tr>
<td>sll1514</td>
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<tr>
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<tr>
<td>slr2076</td>
<td>GroEL</td>
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*The fold change values represent the average of two independent experiments, which were each technically duplicated and additionally subjected to a dye swap. Changes in gene expression less than +/− twofold were not considered to be significant.

encodes the CRP SYCRP1 which binds to the promoter region of slr1667 and directly controls its expression as a transcriptional factor (Yoshimura et al., 2002a). These facts suggest an interesting relationship between cAMP signalling, motility and possible RNA-based regulation, since disruption of *sycrp1* resulted in a non-motile phenotype as well (Yoshimura et al., 2002b). There are different reports suggesting an important role for the second messenger cAMP in motility of *Synechocystis* sp. PCC 6803 cells (Terauchi & Ohmori, 1999, 2004). Mutant colonies lacking the adenylate cyclase Cya1 were impaired in forming typical finger-like projections during phototactic movement, whereas motility was not affected at the individual cell level (Bhya et al., 2006). The former could be reversed by the addition of cAMP to the medium, though not leading to the restoration of motility in *sycrp1* mutant cells. Similarly, in this work, non-motile Δhfq mutant cells were also not rescued by cAMP addition (data not shown). However, there seems to be an important difference between Δsycrp1 and Δhfq mutant cells, that is to say motility of *sycrp1* disruptants is affected only in the ‘strong’ phototaxis characterized by projections on the agar plate, while individual cells still appear motile (Bhya et al., 2006).

Here, it is interesting to draw a comparison with enterobacteria, since the cAMP–CRP complex in *E. coli* is required for flagellum synthesis, besides its involvement in the regulation of several catabolic functions (Botsford & Harman, 1992). Moreover, transcription of the Hfq-dependent sRNAs Spot42 and CyAR in *E. coli* and *Salmonella*, respectively, is controlled by the cAMP–CRP complex (Papenfort et al., 2008); there is evidence that cyaR transcription is under direct control of cAMP and CRP (Papenfort et al., 2008). However, given that cAMP supply could not restore motility in *Synechocystis* sp. PCC 6803 Δhfq, we suppose that Hfq acts epistatically to cAMP-SYCRP1 and probably interferes with a very late step of a signalling chain that regulates cellular movement.

Another interesting aspect arising from the Northern blot data is the apparent involvement of Hfq in accumulation of *spkA* and pilB1 mRNAs, encoding a serine/threonine protein kinase and a putative ATPase, respectively, and also in control of the abundance of pilA1, encoding the major structural component of pili; all these genes are essential for motility (Kamei et al., 2001; Yoshihara et al., 2001; Panichkin et al., 2006). In line with the chosen expression cut-off and statistical stringency, these genes were not identified in the course of the microarray analysis. Interestingly, we found that both *spkA* and *pilB1* became moderately activated in the absence of Hfq, whereas a decrease in pilA1 expression levels in the Δhfq mutant was seen. Intriguingly, the inverse change in expression levels of pilA1 and *spkA* in Δhfq complies with the observation that pilA1 expression is enhanced in a ΔspkA mutant (Panichkin et al., 2006). In contrast, the expression level of the slr2015–2018 operon was lowered in both Δhfq and ΔspkA mutants (Panichkin et al., 2006), together suggesting that the Hfq-dependent signalling pathway is different from SpkA-dependent motility control.

Hfq also appears to be involved in the assembly and functionality of extracellular appendages in other groups of bacteria. We note that there is – irrespective of the completely different lifestyles of pathogens and cyanobacteria – an analogy between pathogenicity and (phototactic) motility in terms of the dependence of both phenomena on such cellular appendages and adherence (reviewed by Bhaya, 2004; Nudelman & Kaiser 2004; Pizarro-Cerdá & Cossart, 2006). Although cell surfaces were not further examined, twitching and swarming mediated by type IV pili were found to be impaired in a Δhfq mutant of
Pseudomonas aeruginosa (Sonnleitner et al., 2003). In a Δhfq mutant of *Salmonella typhimurium*, impaired cell motility correlated with a reduction of flagellin (FliC) expression and drastically reduced virulence (Sittka et al., 2007). However, the structural and mechanistic features of this type of dynamic cell surface appendages are completely different from those of cyanobacterial type IV pili (Bardy et al., 2003). Hence, the functions of the cyanobacterial Hfq orthologue cannot readily be inferred from data for other bacteria.

Certainly, sRNAs are frequently involved in Hfq-dependent regulatory processes in bacteria (reviewed by Storz et al., 2004; Gottesman, 2005; Majdalani et al., 2005), and here we provide the first support for the idea that riboregulatory processes play a role in the control of motility in cyanobacteria. Nevertheless, at present we do not have a clear indication of an RNA chaperone activity of this cyanobacterial Hfq homologue, or of the involvement of a particular sRNA in the regulation of motility. The changes in mRNA levels observed here upon inactivation of *hfq*
Fig. 5. Northern blot-based verification of the genetic complementation of the hfq disruptant. Total RNA was isolated from exponentially growing cultures of the WT and three different mutant strains: Δhfq, disruptant; Δhfq-K, complemented strain harbouring pVZ-Phfq15; Δhfq-S, negative control strain harbouring pVZ-hfqS2, containing an internal stop codon within the hfq ORF. As the hfq knockout had the most striking effect on slr1667 expression, this one was chosen as the representative of hfq-regulated genes.

may not only result from direct binding of Hfq or from disturbed interaction with unknown sRNAs. A comparison of the transcriptome of a Salmonella hfq mutant with the set of Hfq-associated mRNAs has recently shown that the altered transcriptome profile of the mutant can, to some extent, be attributed to secondary effects of the deregulation of transcription factors whose synthesis is directly regulated by Hfq (Sittka et al., 2008).

Preliminary results on isiA and its regulatory antisense-RNA IsrR (Dühring et al., 2006) argue against a role of the cyanobacterial Hfq in riboregulation of photosynthesis; neither of these two transcripts was altered in the Δhfq mutant (data not shown). Arguably, RNA binding remains to be shown for the cyanobacterial Hfq homologue, and associated RNAs with functions in motility control remain to be identified. One strategy that could be used to address these two questions in parallel could be Hfq co-immunoprecipitation, followed by the identification of associated sRNAs (and putative target mRNAs) by microarray analysis (Zhang et al., 2003), direct sequencing (Christiansen et al., 2006) or high-throughput cDNA sequencing (Sittka et al., 2008).

Notwithstanding, the signalling pathway that controls motility in Synechocystis sp. PCC 6803 is clearly more complex than previously thought, integrating Hfq as a new factor. Thus, the functional mechanism(s) of Hfq action in Synechocystis sp. PCC 6803, with special regard to cAMP signalling and motility, as well as potential interaction partners, remains to be investigated and will provide an exciting research area for the future.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft Focus program ‘Sensory and regulatory RNAs in Prokaryotes’ SPP1258 (projects HE 2544/4-1, WI 2014/2-1 and WI 2014/3-1). We thank Gisa Baumert, Ina Wagner, Nils Schürgers, Ingeborg Scholz, Jens Georg and Thomas Wallner for excellent technical assistance or for providing cultures for electron microscopy and Hans Mattijs for access to the microarray design.

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Hfq and cyanobacterial motility


Edited by: D. J. Scanlan