Tn5 transposition in Escherichia coli is repressed by Hfq and activated by over-expression of the small non-coding RNA SgrS

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Abstract

Background: Hfq functions in post-transcriptional gene regulation in a wide range of bacteria, usually by promoting base pairing of mRNAs with trans-encoded sRNAs. It was previously shown that Hfq down-regulates Tn10 transposition by inhibiting IS10 transposase expression at the post-transcriptional level. This provided the first example of Hfq playing a role in DNA transposition and led us to ask if a related transposon, Tn5, is similarly regulated.

Results: We show that Hfq strongly suppresses Tn5 transposition in Escherichia coli by inhibiting IS50 transposase expression. However, in contrast to the situation for Tn10, Hfq primarily inhibits IS50 transposase transcription. As Hfq does not typically function directly in transcription, we searched for a transcription factor that also down-regulates IS50 transposase transcription and is itself under Hfq control. We show that Crp (cyclic AMP receptor protein) fits these criteria as: (1) disruption of the crp gene led to an increase in IS50 transposase expression and the magnitude of this increase was comparable to that observed for an hfq disruption; and (2) Crp expression decreased in hfq−. We also demonstrate that IS50 transposase expression and Tn5 transposition are induced by over-expression of the sRNA SgrS and link this response to glucose limitation.

Conclusions: Tn5 transposition is negatively regulated by Hfq primarily through inhibition of IS50 transposase transcription. Preliminary results support the possibility that this regulation is mediated through Crp. We also provide evidence that glucose limitation activates IS50 transposase transcription and transposition.

Keywords: Tn5/IS50, Hfq, Crp, SgrS, DNA transposition

Background

Transposase proteins catalyze the chemical steps in bacterial transposition reactions. It follows that the regulation of expression of these genes is a critical feature in dictating the transposition frequency of most transposons. In many instances, including Tn10/IS10 and Tn5/IS50, transposase gene promoters are inherently weak. In addition, DNA adenine methylase (DAM) limits initiation of IS10 and IS50 transposase gene transcription by methylating promoter elements [1,2]. These factors together make transcription initiation a limiting step in Tn10/IS10 and Tn5/IS50 transposition reactions [3,4]. There are also examples where translation of transposase transcripts is subject to both intrinsic and host levels of regulation. In the case of IS10 transposase, the ribosome binding site is inherently weak and the transposon encodes an antisense RNA that binds the translation initiation region (TIR), blocking ribosome binding [5,6]. There is also evidence that the ‘host’ protein Hfq helps mediate the pairing interaction between the antisense RNA and the IS10 transposase transcript [7,8].

Hfq is a global regulator of gene expression in bacteria. It typically functions at the post-transcriptional level, influencing translation initiation and/or transcript stability by catalyzing the pairing of small RNAs (sRNA) and their mRNA targets (Figure 1B and reviewed in [9]). In contrast to the many examples of Hfq acting in a post-transcriptional capacity to impact gene expression, there is (to our knowledge) only one example in the literature of Hfq acting at the level of transcription to influence gene expression. In the case of ribosomal proteins rpsO, rpsT and rpsB-tsf, Hfq was shown to increase transcript
levels without influencing transcript stability. It was suggested that this is accomplished through Hfq binding to secondary structure elements in the respective transcripts that form early in the elongation phase of transcription and that this interaction reduces RNA polymerase pausing [10].

As noted above, Hfq has been implicated in the regulation of Tn10/IS10 transposition. Under conditions of hfq deficiency, a large increase in both Tn10/IS10 transposition (up to 80-fold) and transposase expression (up to 7-fold) were observed. The existing evidence is consistent with Hfq acting as a negative regulator of IS10 transposase expression by both antisense dependent and independent pathways. In support of the latter, it was found that hfq deficiency (or h fq ) had a significant impact on Tn10 transposition even when the level of antisense RNA was insufficient to impact on transposase expression (that is when Tn10 is present in single copy in the bacterial chromosome). In addition, there was a synergistic increase in transposase expression when both hfq and the antisense RNA were knocked out, implying that Hfq does not function exclusively in the same pathway as the antisense RNA [7].

Taking the above results into account, and considering that most bacterial transposition systems are not regulated by antisense RNAs, we wondered if Hfq might play a more general role in regulating transposition systems. In the current work, we tested this hypothesis by asking if Tn5 transposition is also regulated by Hfq. Like Tn10, Tn5 is a composite transposon (Figure 1A). The two transposons are closely related but Tn5 lacks an antisense RNA regulatory system and consequently if Hfq were to regulate this system at the post-transcriptional level, it is likely that a trans-encoded sRNA would play a role [11-13]. Tn5 does encode an inhibitor protein that limits Tn5/IS50 transposition by dimerizing with the transposase protein, forming an inactive complex [14]. Transposase and the inhibitor protein are expressed from overlapping promoters, P1 and
P2 (color coded in Figure 1A), with the inhibitor transcript (T2) being expressed at a higher level than the transposase transcript (T1). T1 expression is down-regulated by DAM (reviewed in [15]). There is some evidence that P1 is also negatively regulated by LexA, an SOS-inducible transcriptional repressor [16]. However, there is little else known with regard to host proteins that influence either transposase transcription or translation.

In the current work, we show that both Tn5 transposition and IS50 transposase expression increase significantly in E. coli under conditions of hfq deficiency. However, unlike the situation in Tn10/IS10 transposition, the up-regulation of IS50 transposase expression appears mainly to be due to an increase in transposase gene transcription. As Hfq does not typically function directly in transcription, we looked at the possibility that Hfq regulates IS50 transposase expression by controlling the expression of a transcription factor. Towards this end, we provide evidence that Hfq acts in a regulatory network with Crp (cyclic AMP receptor protein) to down-regulate IS50 transposase transcription. Finally, we demonstrate that over-expression of an sRNA (SgrS) activates expression of the IS50 transposase gene specifically when cells are grown with glucose as the sole carbon source. Evidence is presented that this up-regulation is a consequence of glucose limitation, demonstrating that the IS50 transposase promoter (and Tn5 transposition) is responsive to the nutrient status of the cell.

**Results**

**Hfq is a potent negative regulator of Tn5 transposition**

We asked if Hfq regulates Tn5 transposition in E. coli by measuring the frequency of Tn5 transposition under conditions of hfq deficiency using the ‘mating out’ assay. In this assay, an F’ donor strain harboring a chromosomal copy of Tn5 was mated to an F’ recipient strain and the mating efficiency and number of transposition events were measured by plating mating mixes on the appropriate selective media (see Methods). We show in Figure 2A that in one donor strain background (DBH179) Tn5 transposition increased by close to 75-fold under conditions of hfq deficiency. Note that we did not have a defective copy of Tn5, as transcription was reduced approximately 45-fold relative to when no hfq was present (Figure 2A). Furthermore, plasmid-encoded variants of hfq, including K56A and Y25A, which are impaired for RNA-binding at the ‘proximal’ and ‘distal’ surface, respectively, failed to complement hfq deficiency [17]. This confirms that specific functions of Hfq, namely interaction with RNA via known RNA-binding surfaces, are required for effective repression of Tn5 transposition.

We also tested the impact of hfq deficiency on Tn5 transposition in a second donor strain background (DBH261) via the ‘mating out’ assay (Figure 2B). In this experiment hfq” also caused an increase in Tn5 transposition, although the magnitude of the effect was smaller (approximately 9-fold) than reported for the DBH179 strain background.

**IS50 transposase expression increases in hfq” cells**

We next asked if hfq status influenced IS50 transposase expression. In one approach, we measured transposase expression by constructing IS50-lacZ transcriptional and translational fusions (TCF and ‘TLF’, respectively; see Figure 3A for schematics), integrating these reporters into the chromosome of a lacE E. coli strain (DBH107), and then performing β-galactosidase assays. This was done for each reporter in isogenic strains that were either wt, dam” or hfq”. As expected for a promoter that is DAM-sensitive, transposase expression increased in the context of both transcriptional and translational fusions in the dam” strain relative to wt (approximately 19- and 25-fold, respectively; Figure 3B). The increase in transposase expression for both constructs in dam” is indicative of expression coming predominantly from the P1 promoter [2]. Transposase expression in TCF and TLF constructs also increased in hfq” cells (11-fold and 7.4-fold, respectively), indicating that Hfq (or a factor under Hfq control) represses IS50 transposase expression. As the TCF encodes only 15 nucleotides of the transposase transcript (T1), it seemed most likely that up-regulation of transposase expression in hfq” was primarily due to enhanced transcription in both TCF and TLF constructs.

To further test this possibility, we constructed a TLF reporter (pDH908) wherein the IS50 transposase promoter was replaced by a heterologous promoter (from the lpp gene) whose regulation is not sensitive to hfq status [10]. An isogenic plasmid (pDH795) in which the TLF contained the IS50 transposase promoter was also constructed. Cells (wt or hfq”) were transformed with plasmids containing these constructs and reporter expression was measured as above. The results presented in Figure 4 show that transposase expression increased approximately 9-fold for the construct containing the IS50 promoter and less than 2-fold for the construct
containing the lpp promoter. These results support our contention that Hfq-directed regulation of IS50 transposase expression occurs at the transcriptional level because the absence of the IS50 promoter and not the presence of the IS50 5’ UTR was the dominant factor in observing strong up-regulation of reporter expression under conditions of hfq deficiency.

Hfq impacts steady-state levels of full-length IS50 transposase mRNA

To further assess the impact of hfq deficiency on transposase gene expression, we looked at both the steady-state level and the stability of the transposase transcript (T1) in hfq+ and hfq− cells. For the steady-state analysis, total RNA was isolated from various strains (wt or hfq−).
(DBH33 background) containing a multi-copy plasmid encoding the full-length transposase gene under the control of its native promoter. In addition to the wt version of this plasmid (pDH533), we also analyzed a mutant form containing mutations in the overlapping dam methylation sites in the transposase promoter (pDH752) (see Figure 1A); these mutations make this construct DAM insensitive. Primer extension was used to detect both T1 and T2 transcripts, as well as the lpp transcript (loading control). As expected for a dam-sensitive promoter, levels of T1 increased substantially (approximately 8-fold) in wt cells containing the plasmid with the dam-insensitive promoter versus wt cells containing the wt promoter (compare lanes 3 to 7 with lanes 8 to 12 in Figure 5A and bar graph in Figure 5B). In contrast, there was no significant change in T2 levels in the above samples. In hfq− (wt promoter) there was also a substantial increase in T1 levels (11-fold) versus the wt strain (compare lanes 3 to 7 with lanes 14 to 18) and no significant change in T2 levels. Thus in an hfq− background there was an increase in the steady-state level of transposase transcript and this increase was slightly greater than that observed when methylation of the transposase promoter was blocked.

We also looked at the combined impact of knocking out Hfq and blocking DAM methylation on T1 levels (lanes 19 to 23 in Figure 5A). In comparison to wt, the ‘double mutant’ situation resulted in a 45-fold increase in T1 levels. Based on the observed synergy, we think it unlikely that the observed impact of deleting hfq is linked to the regulation of dam expression.

To directly test if a component of Hfq-directed repression of IS50 transposase expression is post-transcriptional,
we compared the stability of the IS50 transposase mRNA (T1) in isogenic wt and hfq− strains. Total RNA was isolated from a pair of rifampicin-sensitive strains (TM338 and TM618) containing a plasmid encoding IS50 transposase (pDH533) before and after rifampicin treatment as shown in Figure 6. Transposase mRNA was detected by primer extension. In the hfq− strain the half-life of the T1 transcript increased by approximately 1.7-fold, revealing that hfq status does impact on transposase mRNA stability. Taken together, the results from Figures 3, 4, 5 and 6 show that IS50 transposase expression is substantially reduced in an hfq+ relative to an hfq− strain and that hfq status primarily affects transposase transcription.

Regulation of Tn5 transposase expression by global transcriptional regulators

As Hfq does not typically function directly in transcription, we set out to define a transcription factor that down-regulates IS50 transposase transcription and is itself regulated by Hfq. Toward this end, we asked if disrupting genes for two global transcription factors, Crp and Lrp [18], had an impact on IS50 transposase expression. For example, in cells grown in exponential phase in Luria broth (LB), there was up-regulation of transposase expression (approximately 4-fold) in both crp− (DBH307) and hfq− (DBH306) strains but not in the lrp− strain (DBH315). We also performed semi-quantitative RT-PCR and show that transposase-lacZ transcript levels increased similarly in crp− and hfq− strains (Figure 7B). These results are consistent with Crp being a negative regulator of IS50 transposase transcription.

We next asked if Crp expression was regulated by Hfq. Notably, work done in Yersinia pestis has shown that Hfq positively regulates Crp expression at the post-transcriptional level [20]. Towards this end we performed Western blot analysis with a Crp antibody on E. coli cell extracts from wt (DBH303), hfq− (DBH306) and crp− (DBH307) strains (Figure 7C). The results show that lower levels of Crp are present in the hfq− strain, which is consistent with Hfq also being a positive regulator of Crp expression in E. coli.
Finally, we assessed the impact of knocking out _crp_ on _Tn5_ transposition frequency using the ‘mating out’ assay (Figure 7D). In the absence of _crp_, _Tn5_ transposition increased 7-fold, which is consistent with results from the transposase expression experiments.

**IS50 transposase expression and _Tn5_ transposition are up-regulated by over-expression of the sRNA _SgrS_**

Over-expression of sRNAs can alter Hfq-regulated networks by limiting the availability of Hfq [21,22]. Given our findings that _Tn5_ transposition and transposase gene expression are affected by _hfq_ status, we asked if _IS50_ transposase expression might be sensitive to Hfq-titration. Towards this end, we measured transposase expression from the TLF under conditions where a single sRNA was over-expressed from an IPTG inducible promoter (pDH533) and total RNA was isolated either before or after the addition of rifampicin (at the indicated time points). Transposase RNA was detected as described in Figure 5. The bands were quantified (ImageQuant) and T1 normalized to un-extended primer before plotting the proportion of RNA remaining after rifampicin addition (time zero = 1.0). The data was fit to a one-phase exponential decay curve by non-linear regression (Prism) to determine the half-life (t1/2). The data shown is a compilation from two independent experiments.

**Figure 6** _IS50_ transposase mRNA half-life analysis. Strains TM338 (wt) and TM618 (hfq −) were transformed with _IS50_ transposase encoding plasmid pDH533 and total RNA was isolated either before or after the addition of rifampicin (at the indicated time points). Transposase RNA was detected as described in Figure 5. The bands were quantified (ImageQuant) and T1 normalized to un-extended primer before plotting the proportion of RNA remaining after rifampicin addition (time zero = 1.0). The data was fit to a one-phase exponential decay curve by non-linear regression (Prism) to determine the half-life (t1/2). The data shown is a compilation from two independent experiments.

Given the comparable Hfq binding affinities of the sRNAs tested, it seemed unlikely that _SgrS_ expression was increasing transposase expression through an Hfq-titration mechanism.

_SgrS_ down-regulates the expression of several known targets, including the primary glucose transporter encoded by the _ptsG_ gene, a mannose transporter encoded by _manXY_ and it up-regulates the expression of _yigL_, a phosphatase involved in phospho-sugar detoxification [26]. As we observed up-regulation of _IS50_ transposase expression in cells over-expressing _SgrS_ in M9 glucose media, we considered the possibility that this effect was a response to glucose limitation. In fact, we show in Additional file 2 that induction of _SgrS_ in M9 glucose resulted in a substantial slowing of bacterial growth, as would be expected if nutrients had become growth-rate limiting. To further test the glucose limitation hypothesis, we performed a similar experiment in rich media (LB) and in M9 glucose supplemented with glycerol, a carbon source whose import is not dependent on glucose transporters [27]. We also tested the response of the reporter to over-expression of an _SgrS_ mutant, _sgrS1_, that is incapable of down-regulating glucose import [28]. In these experiments we used a _Tn5_ TCF as a reporter in the DBH107 strain background; DBH107 has a complete deletion of the lac operon and consequently the plasmid-encoded sRNA genes are constitutively expressed. To avoid problems in growing these cells, cultures were initially propagated in either LB or M9 glucose/glycerol and then were propagated in either LB or M9 glucose/glycerol and then where indicated, switched to other media.

We show in Figure 8B that after approximately 4 hours of _SgrS_ over-expression in M9 glucose, reporter expression increased close to 5-fold relative to a ‘vector’ control. In contrast, over-expression of _SgrS1_ was incapable of up-regulating reporter expression under these same conditions, suggesting that _SgrS_ must be able to down-regulate glucose import and or retention in order to increase transposase transcription. When cells were grown in M9 glucose supplemented with glycerol, expression of _SgrS_ as above caused only an approximately 2-fold increase in transposase expression. Importantly, the reduced effects of _SgrS_ on transposase expression under ‘glycerol’ conditions cannot be explained by differential expression of the respective sRNAs, as levels of _SgrS_ and _SgrS1_ were similar in M9 glucose with or without glycerol (Figure 8C). Also, we failed to see significant transposase induction when _SgrS_ was over-expressed in LB media where there are multiple carbon sources. Finally, consistent with the glucose limitation hypothesis, we also show in Figure 8B that increased transposase expression resulting from _SgrS_ expression in M9 glucose was the only condition that inhibited cell growth.

Given that transposition frequency is expected to be roughly proportional to transposase expression, we also asked if glucose limitation had an impact on _Tn5_
transposition. Cells encoding a chromosomal copy of Tn5 were transformed with an SgrS-expressing plasmid (or vector only control) and the frequency of Tn5 transposition was measured using the ‘mating out’ assay. Note that cells were grown in M9 glucose media and SgrS expression was induced only when donor strains were subcultured on the day of mating. We show in Figure 9 that induction specifically of SgrS resulted in a 5-fold increase in Tn5 transposition relative to the vector only control. Notably, when cells were grown in M9 supplemented with glucose and glycerol, induction of SgrS did not result in a significant increase in Tn5 transposition. Also, we observed a reduced growth rate only in cultures where SgrS was induced in M9 glucose media (data not shown). The results of the ‘mating out’ analysis are thus entirely consistent with the gene expression experiments presented in Figure 8.

**Discussion**

Hfq is a global regulator of gene expression in bacteria. However, until recently, Hfq had not been linked to the control of transposable elements. Work in the Tn10/IS10 system provided the first example of Hfq inhibiting a transposon [7]. In the current work, we asked if the transposition of a related element, Tn5/IS50, is also regulated by Hfq. We show that Tn5 transposition and IS50 transposase expression are repressed by Hfq; however, the mechanism of repression is atypical for Hfq, involving predominantly a block in IS50 transposase transcription. Preliminary evidence is presented that is consistent with Hfq modulating IS50 transposase transcription through regulation of Crp. We also show that transposase transcription and Tn5 transposition are activated by over-expression of the sRNA SgrS and provide evidence that this is a transcriptional response to glucose limitation.

**Hfq negatively regulates Tn5 transposition**

The results of ‘mating out’ experiments were consistent with Hfq acting as a strong negative regulator of Tn5 transposition. Tn5 transposition increased close to 75-fold...
in one \(hfq\textsuperscript{−}\) strain (DBH179 background). The magnitude of this increase was somewhat surprising given that up-regulation of \(Tn10\) in \(hfq\textsuperscript{−}\), under essentially antisense-minus conditions, was about 7-fold [7]. However, in a different \(hfq\textsuperscript{−}\) strain (DBH261 background) \(Tn5\) transposition increased only 9-fold. At this point it is unclear why there was such a large discrepancy in the ‘mating out’ values for the two strains. One possibility is that colony counts in the DBH179 ‘mating out’ (\(hfq\textsuperscript{−}\)) included clones that had ‘jack-pot’ events. That is, colonies were counted that did not derive from independent transposition events. This could explain the high standard error associated with the transposition frequency in the \(hfq\textsuperscript{−}\) column in Figure 2A.

If, for example, we removed the 3 most prominent outliers from the (DBH179) \(hfq\textsuperscript{−}\) data set, the fold increase in transposition dropped to 15-fold, which is more in line with what we observed in the DBH261 strain background and for \(Tn10\) in single copy [7].
A trans-complementation (Figure 2A) experiment provided definitive proof that the increase in Tn5 transposition detected in one of our hfq" mating out strains (DBH179 background) was in fact due to hfq deficiency. In addition, the failure of two Hfq RNA-binding face mutants to provide complementation was consistent with Hfq-directed inhibition of Tn5 transposition relying on functions of Hfq required in canonical Hfq-directed regulatory pathways [17]. That is, Hfq must retain the ability to bind both mRNAs and sRNAs to influence Tn5 transposition.

Hfq, Crp and IS50 transposase gene expression

Evidence that hfq status influences IS50 transposase expression came from two types of experiments. First, the expression of transposase-lacZ reporter genes in both transcriptional and translational fusion constructs increased significantly under conditions of hfq deficiency. Second, the steady-state level of the native transposase creased significantly under conditions of transcriptional and translational fusion constructs in transposition.

That is, Hfq must retain the on functions of Hfq required in canonical Hfq-directed inhibition of the level of transcription. Notably the suppressive effect of Hfq on IS50 transposase transcription was remarkably specific, as the level of a second transcript (T2) encoded by IS50 was not affected by hfq status.

As Hfq does not typically act directly in gene transcription, we think it likely that Hfq acts indirectly on the IS50 transposase promoter. In addition to DAM, only one other transcription factor, LexA, has been implicated as a regulator of transposase transcription. There is a weak LexA-binding site in the transposase promoter (Figure 1A); however, lexA deficiency was shown to increase transposase transcription only to two to three-fold in a TCF [16]. As we have seen increases in transposase expression of up to 11-fold for a TCF in hfq−, it seems unlikely that Hfq would be working through LexA. In contrast, transposase expression increased in dam− to a level more in line with that observed in hfq− (less than two-fold difference in the TCF). However, the observed synergy between hfq− and mutations that rendered the IS50 transposase promoter DAM-insensitive led us to conclude that Hfq does not regulate IS50 transcription by impacting DAM levels (and, therefore, promoter methylation). These results provided motivation to search for other targets of Hfq that impinge on IS50 transposase transcription. This search identified Crp as an additional negative regulator of IS50 transposase transcription. Notably, transposase expression increased to approximately the same level in crp− and hfq− in the experiment in Figure 7. The similar magnitude of up-regulation of transposase expression in hfq− and crp− could be indicative of Hfq acting upstream of Crp to inhibit transposase expression. We did in fact find evidence of Hfq positively regulating Crp protein levels (Figure 7C). This observation is consistent with work recently published in the Y. pestis system where it was found that Crp protein levels decreased approximately five-fold in an hfq disruption strain [20].

Crp is a known activator/repressor of transcription [18] and, therefore, more likely than Hfq to be directly involved in regulating IS50 transposase expression at the transcriptional level. Given our evidence that Hfq positively regulates crp expression, a plausible scenario explaining our expression data is that the observed up-regulation of IS50 transposase transcription in hfq− is a result of decreased Crp protein levels. Crp may act either directly or indirectly on the IS50 transposase promoter to repress transcription. This is currently a working model as we have not yet tested the possibility that Crp binds the IS50 transposase promoter and it may only be coincidental that transposase expression increased to similar levels in hfq− and crp− strains. Notably, we also found that Tn5 transposition increased when the crp gene was disrupted, although the extent of the increase was smaller than that observed in the isogenic hfq disruption strain. This could be indicative of additional factors in the Hfq regulon impinging on Tn5 transposition.

There is precedent for Crp down-regulating the transcription of a transposase gene. In the case of IS2,
transposase transcription increased close to 200-fold in \texttt{crp} \textsuperscript{-}. It was also shown through protein-DNA footprinting that Crp binds directly to the IS2 transposase promoter [29]. Interestingly, based on the consensus binding sequence for Crp, the authors of the above study predicted that Crp would bind to the \texttt{ISSO} transposase gene. However, the predicted \texttt{crp} binding site is located downstream of the transposase promoter and is not present in our TCF (where we detected increased transposase expression in \texttt{crp} \textsuperscript{-}). Nevertheless, it would be worthwhile to test for Crp binding to the \texttt{ISSO} transposase promoter as the results of Crp ChIP-chip studies revealed the presence of thousands of weak \texttt{crp} binding sites scattered throughout the \textit{E. coli} genome [30]. It is also possible that Crp acts indirectly on the \texttt{ISSO} transposase promoter by regulating the expression of an other transcription factor.

\textbf{Tn5 transposition and metabolic stress}

We also identified conditions that activate transposase expression and transposition; over-expression of the sRNA SgrS increased transposase expression and transposition approximately five-fold. We favor the possibility that this induction is a consequence of glucose limitation but cannot formally rule out the possibility that SgrS targets an as yet undefined regulatory pathway that impinges on transposase expression. Our reasoning for this is that we observed induction of transposase expression and transposition specifically when cells were grown with glucose as the major carbon source and SgrS is known to prevent expression and function of the major glucose transporter encoded by the \texttt{ptsG} gene [26]. Consistent with this idea, we found that transposase induction levels correlated with a reduced growth rate. Furthermore, we demonstrated that: (i) an allele of SgrS (\texttt{sgrS1}) that is incapable of down-regulating \texttt{ptsG} expression failed to induce transposase expression in M9 glucose; (ii) under conditions where SgrS was expressed in M9 glucose media supplemented with glycerol, we failed to see induction of transposase expression to the same extent as when glycerol was absent; (iii) SgrS expression did not impact transposase expression when cells were grown in rich media (LB) and (iv) over-expression of 3 other sRNAs (RybB, RyeB and MicC) that are not expected to influence glucose transport did not increase transposase expression in M9 glucose [31-33]. Precedent for nutritional stress influencing transposition comes from earlier work in the \textit{IS903} system where mutations in a gene (\texttt{aspa4}) required for fermentative metabolism during anaerobic growth caused transposition to occur at an accelerated rate [34].

At this point it is unclear as to what factors are driving the induction of the transposase gene under SgrS over-expression conditions. With regard to further defining the mechanism of \texttt{ISSO} transposase up-regulation under SgrS over-expression conditions, it would also be advantageous to find alternative experimental conditions for achieving this increased expression. If, for example, simply starving cells by restricting a carbon source during growth achieves the same end as over-expressing SgrS in M9 glucose media, an unbiased screen to search for genetic factors that are necessary for the up-regulation of transposase expression could be performed to reveal the regulatory network impinging on the transposase promoter. As it stands, any factors that influence SgrS expression would interfere with the outcome of such a screen. Alternatively, if it was found that restricting glucose is not sufficient for inducing transposase expression, the possibility that SgrS plays a more direct role in controlling transposase expression would have to be considered.

\textbf{Conclusions}

In this work, we have identified several genes that impact on \texttt{ISSO} transposase expression, including \texttt{hfq}, \texttt{crp} and \texttt{sgrS}. Hfq and Crp proteins are negative regulators and SgrS RNA (under specific growth conditions) is a positive regulator of transposase gene expression. Exactly how these factors impinge on transposase expression remains to be worked out and at this point it is not clear if we are seeing modulation of the same regulatory network in opposite directions when \texttt{hfq} and \texttt{crp} genes are disrupted and SgrS RNA is over-expressed. \texttt{Tn5/ISSO} is the second transposon identified that is affected by disruption of the \texttt{hfq} gene and the first that does not encode an antisense RNA. This raises the possibility that Hfq influences the transposition frequency of many other bacterial transposons.

\textbf{Methods}

\textbf{Plasmids, bacteriophage and strains}

The \texttt{ISSO} translational fusion plasmid (pDH798) is a pWKS30-derivative containing base pairs 1 to 431 of \texttt{IS50} transposase transcriptional fusion plasmid (pDH798) is a pWKS30-derivative containing base pairs 1 to 431 of \texttt{IS50} transposase transcriptional fusion plasmid (pDH682) is a pUC18-derivative containing base pairs 1 to 80 of \texttt{ISSO} (nucleotides 1 to 15 of T1) fused to nucleotide -16 (relative to the translational start codon) of \texttt{lacZ}. Plasmids encoding sRNAs (pDH764, sgrS; pDH766, rybB; pDH768, micC; pDH772, ryeB) and the corresponding empty vector control (pDH763) were kindly provided by S Gottesman. The plasmid encoding \texttt{sgrS1} (pDH895) was kindly provided by C Vanderpool. Plasmids encoding Hfq (pDH700, wt) and mutant derivatives (pDH701, K56A; pDH713, Y25A) are described in Ross et al [8]. Details of plasmid constructions are provided in Additional file 3 and a list of oligonucleotides used in this work is provided in Additional file 4.

Lambda phages encoding \texttt{ISSO} transcriptional (\texttt{λDBH849} and \texttt{λDBH888}) and translational (\texttt{λDBH812}) reporters were generated by cloning \texttt{ISSO} expression cassettes marked with
an antibiotic resistance gene (either kan<sup>R</sup> or cm<sup>R</sup>) into the his operon of pNK81 and then infecting a strain harboring one of these plasmids with λNK1039, which also contains the his operon. Antibiotic resistant lysogens from the above crosses were selected by replica plating and subsequently phage released from the lysogens were purified, giving rise to λDBH849 (IS50-lacZ-kan<sup>R</sup> TCF), λDBH888 (IS50-lacZ-Cm<sup>R</sup> TCF) and λDBH812 (IS50-lacZ-Kan<sup>R</sup> TLF).

E. coli strains for the ‘mating out’ assay were constructed by P1 transduction of Tn5 from ER2507 (NEB) into DBH33, DBH344 and DBH259. Strains containing chromosomal IS50-lacZ fusions were generated by lysogenizing DBH107 with λDBH849 (DBH265), λDBH888 (DBH303) or λDBH812 (DBH281). Mutant derivatives of these strains were generated by P1 transduction. A list of all of the strains, plasmids and bacteriophage used in this work is presented in Table 1.

‘Mating out’ assay
Conjugal ‘mating out’ experiments were performed essentially as described for single-copy chromosomal transposons in Ross et al. [7], except that for measuring transposition in hfg<sup>−</sup> versus wt, donor growth was carried out in M9 glucose media supplemented with kanamycin (25 µg/mL) and amino acids, instead of LB. DBH13 was used as the recipient. Total exconjugants and transposition events with DBH107 and derivatives were scored by plating mating mixes on M9 glucose plates supplemented with leucine, thiamine and streptomycin (150 µg/mL) or streptomycin and kanamycin (25 µg/mL), respectively. Total exconjugants and transposition events with DBH261 and derivatives were scored by plating mating mixes on M9 glucose plates supplemented with leucine, thiamine, streptomycin (150 µg/mL) and gentamicin (12.5 µg/mL) or streptomycin, gentamicin and kanamycin (25 µg/mL), respectively.

β-galactosidase assays
Cells were grown in M9 glucose (with arginine and thiamine) or LB. In situations where strains contained plasmids, plasmids were maintained by including the appropriate antibiotic. Overnight cultures (0.05 mL) were used to seed subcultures (1.5 mL), which typically were grown to mid-log phase before being processed for the Miller assay as previously described [7].

RNA isolation, primer extension and Northern blot analysis
Total RNA was isolated essentially as described in [50]. For steady-state analysis, cells were grown to mid-log phase in LB before RNA isolation. For half-life analysis, rifampicin (dissolved in dimethyl sulfoxide; DMSO) was added to cell cultures (to 200 µg/mL) to arrest transcription and RNA was isolated immediately before and after rifampicin addition at the indicated time intervals. Primer extension analysis was carried out using <sup>32</sup>P-labeled primers oDH230 and oDH390, end-labeled with OptiKinase (USB, Cleveland, OH, USA) according to manufacturer’s instructions. Extension reactions used 5 µg of RNA, and Superscript III reverse transcriptase essentially as described in [51], except that annealing was performed at 65°C (with no ice treatment) before extending at 55°C for 45 minutes. Extension products were resolved on 6% and 10% denaturing polyacrylamide gels. For Northern blot analysis, 2 µg of RNA was mixed with an equal volume of denaturing loading dye (95% deionized formamide [v/v], 10 mM EDTA, 0.5× TBE, 3% xylene cyanol [w/v]), heated to 95°C for 2 minutes, and resolved on a 6% polyacrylamide gel containing 7 M urea. Separated RNAs were electro-transferred to Hybond N (GE Healthcare, Mississauga, ON, Canada) in 0.5× TBE and fixed with UV. Annealing and washing was performed in ULTRAhyb buffer (Ambion, Burlington, ON, Canada) according to the manufacturer’s instructions, using RNA probes complimentary to SgrS or the 5S rRNA (internal standard). To construct the radiolabeled RNA probes, DNA templates for in vitro transcription were made by PCR with primers oDH323/233 (SgrS) and oDH234/235 (5S rRNA) - note that, for each primer pair, the forward primer includes the T7 core promoter. These templates were transcribed in vitro in the presence of <sup>32</sup>P-UTP to generate uniformly labeled RNA probes. In vitro transcription reactions were performed in 25 µL volumes with approximately 1 µg DNA template, 1 × T7 RNA polymerase buffer (NEB, Beverly, MA, USA), 20 units RNAsin (Promega, Madison, WI, USA), 4 mM dithiothreitol (DTT), 0.16 mg/mL BSA, 0.4 mM each of GTP, CTP and ATP, 0.01 mM UTP, 50 µCi [α-<sup>32</sup>P]UTP, and 100 units of T7 RNA polymerase.

Western blot
Cells were centrifuged (2 minutes at 21,000 × g), resuspended in SDS load mix (2% [w/v] SDS, 10% [v/v] glycerol, 50 mM Tris-HCl pH 6.8, 0.25% [w/v] bromophenol blue, 0.8 M β-mercaptoethanol) and heated at 95°C for 5 minutes. To normalize for differences in growth between the various samples, the OD<sub>600</sub> of each sample was measured and the volume spun normalized to give an equivalent to OD<sub>600</sub> approximately equal to 0.35. The resulting lysates were subjected to SDS-PAGE on a 12% polyacrylamide gel, proteins transferred to PVDF (Roche, Indianapolis, IN, USA) and Crp was detected by Western blot with a polyclonal rabbit anti-Crp antibody (kind gift of H Aiba). The primary antibody was diluted 1:20,000 in TBST; the secondary antibody (anti-rabbit IgG-horseradish peroxidase (HRP) conjugate; Promega, Madison, WI, USA) was used at 1:5,000. Crp was visualized with a Pierce ECL 2 Western blotting substrate (Thermo Scientific, Rockford, IL, USA) and PhosphorImager (GE Healthcare). The membranes were stripped and GroES detected (rabbit anti-GroES
### Table 1: Plasmids, bacteriophage and strains

| Strain or Plasmid | Relevant genotype | Use | Source or reference |
|-------------------|-------------------|-----|---------------------|
| **E. coli**        |                   |     |                     |
| DBH13             | HB101 [F- leu-pro ]; StrR | 'Mating out' recipient | [36] |
| ER2507            | zjc::Tn5; KanR | Source of zjc::Tn5 | NEB |
| DBH179            | NKS830 [recA- argF lacpro] zjc::Tn5; KanR | 'Mating out' donor | This study |
| DBH184            | DBH179 hfq-1::Ωcat; KanR; CmR | 'Mating out' donor | This study |
| DBH228            | R2211/pOX38Gen | Source of pOX38Gen | [37] |
| DBH233            | HW-5 [phoA4(Am) his-45 recA- rpsL; StrR | Parent strain | [38] |
| DBH259            | DBH233/pOX38Gen; StrG | Parent strain | This study |
| DBH261            | DBH259 zjc::Tn5; StrG K | 'Mating out' donor | This study |
| DBH265            | DBH107/ADP849; StrR | Miller Assay | This study |
| DBH267            | DBH265 hfq-1::Ωcat; StrG K | Miller Assay | This study |
| DBH268            | DBH265 dam::Tn9cat; StrG K | Miller Assay | This study |
| DBH281            | DBH107/ADP88; StrR | Miller Assay | This study |
| DBH283            | DBH281 hfq-1::Ωcat; StrG K | Miller Assay | This study |
| DBH285            | DBH281 dam::Tn9cat; StrG K | Miller Assay | This study |
| DBH303            | DBH107/ADP88; StrR | Miller Assay | This study |
| DBH306            | DBH303 Δhfq72::kan; StrG K | Miller Assay | This study |
| DBH307            | DBH303 Δcrp765::kan; StrG K | Miller Assay | This study |
| DBH315            | DBH303 Δcrp77::kan; StrG K | Miller Assay | This study |
| DBH33             | NKS830 [recA- argF lacpro] | Parent strain | [40] |
| DBH16             | DBH33 hfq-1::Ωcat; CmR | Parent strain | [7] |
| DBH241            | DBH33 dam::Tn9cat; CmR; | Parent strain | This study |
| DBH238            | DBH33/ADH849; KanR | Miller Assay | This study |
| DBH239            | DBH238 hfq-1::Ωcat; KanR CmR | Miller Assay | This study |
| DBH240            | DBH238 dam::Tn9cat; KanR CmR | Miller Assay | This study |
| DBH208            | DBH33/ADH812; KanR | Miller Assay | This study |
| DBH210            | DBH208 hfq-1::Ωcat; KanR CmR | Miller Assay | This study |
| DBH237            | DBH208 dam::Tn9cat; KanR CmR | Miller Assay | This study |
| DBH323            | DBH107 recA-; StrR | Miller Assay | This study |
| DBH326            | DBH107 recA- hfq-1::Ωcat; StrR CmR | Miller Assay | This study |
| DBH242            | DBH33 Δcrp765::kan | Parent strain | This study |
| DBH344            | DBH242 Δcrp765; KanR | Parent strain | This study |
| TM338             | W3110mc me-Flag-cat; rifC CmR | RNA half-life measurements | [41] |
| TM618             | W3110mc me-Flag-cat Δhfq; rifC CmR | RNA half-life measurements | [42] |
| DH5Sa             | recA- | Plasmid propagation | Invitrogen |

### Plasmids

| Plasmid | Description | Use | Source or reference |
|---------|-------------|-----|---------------------|
| pWKS30  | pSC101-derived; low copy-number ori | 'Empty vector for Hfq expression' | [35] |
| pDH700  | pWKS30::P3-hfq::Ap | HfqWT expression | [7] |
| pDH701  | pWKS30::P3-hfq::Ap | HfqK56A expression | [7] |
| pDH713  | pWKS30::P3-hfq::Ap | HfqY25A expression | [8] |
Table 1  Plasmids, bacteriophage and strains (Continued)

| Plasmid | Description | Source/Type |
|---------|-------------|-------------|
| pDH633 | pUC18-derivative, Tn5 t'ase M56A; Ap^R | Source of Tn5 transposase (No Inh) [43] |
| pDH752 | pDH533 with t'ase mutated to G53A,C61A; Ap^R | DAM-insensitive t'ase | This study |
| pDH828 | pDH533 with t'ase mutated to D97A; Ap^R | Catalytic t'ase | This study |
| pNK81 | pBR333-derivative; encodes his operon; Ap^R | Lambda crosses | [44] |
| pDH682 | pUC18-derivative; IS50-lacZ TCF; Ap^R | Source of TCF | This study |
| pDH838 | pDH682-derivative; TCF 'marked' with kan^R | Parent of pDH849 | This study |
| pDH883 | pDH682-derivative; TCF 'marked' with cm^R | Parent of pDH888 | This study |
| pDH849 | TCF-kan^R from pDH682 cloned into BclI-cut pNK81; Ap^R | For crossing TCF onto λ | This study |
| pDH888 | TCF-cm^R cloned onto BclI-cut pNK81; Ap^R | For crossing TCF onto λ | This study |
| pDH658 | pRZ9005-derivative; full-length IS50-lacZ TLF; Ap^R | Parent of pDH795 | This study |
| pDH795 | pDH658-derivative; 'deletion' TLF used in this study; Ap^R | Parent of pDH804 | This study |
| pDH804 | pDH795-derivative; TLF 'marked' with kan^R | Parent of pDH812 | This study |
| pDH812 | TLF-kan^R cloned into BclI-cut pNK81; Ap^R | For crossing TLF onto λ | This study |
| pDH753 | pWGS50-derivative; contains IS50-lacZ TLF from pDH658; Ap^R | Parent of pDH798 | This study |
| pDH798 | pDH753-derivative; Ap^R | Miller Assay | This study |
| pDH763 | pBR-plac; Ap^R | Vector for sRNA-induction | [45] |
| pDH764 | pBR-plac-sgrS; Ap^R | SgrS-induction | [46] |
| pDH895 | pBR-plac-sgrS1; Ap^R | SgrS1-induction | [47] |
| pDH766 | pBR-plac-rybB; Ap^R | RyeB-induction | [48] |
| pDH768 | pBR-plac-micC; Ap^R | MicC-induction | [48] |
| pDH772 | pBR-plac-ryeB; Ap^R | RyeB-induction | [48] |
| pDH908 | pDH766-derivative; Lpp-TLF | Miller Assay | This study |

**Phage**

| Phage | Description |
|-------|-------------|
| ANK1039 | Encodes his operon | Parent phage | [49] |
| XD8H812 | IS50-lacZ translational fusion (TLF) from pDH812 marked with kan^R | Chromosomal TLF construction | This study |
| XD8H849 | IS50-lacZ transcriptional fusion (TCF) marked with kan^R | Chromosomal TCF construction | This study |
| XD8H888 | IS50-lacZ transcriptional fusion (TCF) marked with cm^R | Chromosomal TCF construction | This study |

**Antibodies**

Antibody from Sigma-Aldrich (St Louis, MO, USA) at 1:10,000 for use as an internal standard; GroES is not sensitive to hfq status [19]. Bands were quantified using ImageQuant software (GE Healthcare) and Crp levels plotted relative to GroES.

**Additional files**

Additional file 1: Mapping Tn5 transposition events. Southern blot and ST-PCR characterization of Tn5 transposition events in wt and hfq^−^ strains.

Additional file 2: Impact of SgrS over-expression on growth rate in M9 glucose. Growth curves of cells in which SgrS RNA was or was not induced by IPTG addition.

Additional file 3: Details of plasmids constructed for this work.

Additional file 4: List of oligonucleotides used in this work.

**Abbreviations**

BSA: bovine serum albumin; Crp: cyclic AMP-receptor protein; DAM: DNA adenine methylase; DMSO: dimethyl sulfoxide; DTT: dithiothreitol; EDTA: ethylenediaminetetraacetic acid; LB: Luria broth; PAGE: polyacrylamide gel electrophoresis; PCR: polymerase chain reaction; RT-PCR: reverse transcriptase-polymerase chain reaction; sRNA: small RNA; SDS: sodium dodecyl sulfate; TBE: Tris-borate-EDTA buffer; TBST: 20 mM Tris-HCl (pH 7.5), 150 mM sodium chloride, 0.5% Tween-20; TCF: transcriptional fusion; TIR: translation initiation region; TLF: translational fusion; UTR: untranslated region; UV: ultraviolet light.

**Competing interests**

The authors declare that they have no competing interest.

**Authors' contributions**

JR performed 'mating out' assays, β-galactosidase assays in Figures 3, 7 and 8, RT-PCR assays, Western blot assays, participated in the design of the study and helped draft the manuscript. RT performed the β-galactosidase assay in Figure 4, steady-state transcript measurements and RNA half-life measurements and prepared figures. MDB helped construct chromosomal reporter strains. CRM made the initial discovery that Tn5 transposition is up-regulated under conditions of hfq deficiency and constructed IS50-reporter plasmids and expression plasmids. DBH participated in the design of the study, helped in constructing strains and plasmids, performed the 'mating out' experiment in Figure 7D and drafted the manuscript. All authors read and approved the final manuscript.

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