molecular systems biology

Competing pathways control host resistance to virus via tRNA modification and programmed ribosomal frameshifting

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Review timeline:

Submission date:	10 August 2011
Editorial Decision:	03 October 2011
Revision received:	18 November 2011
Editorial Decision:	08 December 2011
Revision received:	14 December 2011
Accepted:	14 December 2011

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 03 October 2011

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the three referees who agreed to evaluate your manuscript. As you will see from the reports below, the referees, while broadly supportive, raised a series of important concerns which will need to be conclusively addressed before this work would be acceptable for publication at Molecular Systems Biology, some of which appear to require additional experimental work (see in particular reviewer #3).

In addition, the reviewers felt that additional data and model details should be provided. Please note, that in addition to our capacity to host datasets in our supplementary information section, we provide a new functionality that allows readers to directly download the 'source data' associated with selected figure panels (e.g. <http://tinyurl.com/365zpej>). This sort of figure-associated data may be particularly appropriate for this work, especially to help address the second Reviewer's concerns regarding Fig. 6. Please see our Instructions of Authors for more details on preparation and formatting of figure source data (<http://www.nature.com/msb/authors/index.html#a3.4.3>).

Similarly, we ask that the mathematical models presented in this work be provided in a common machine-readable format with any revised work (SMBL is strongly encouraged, but you may wish to submit the actual Matlab source code in addition), and we request that models be submitted to a public repository like BioModels whenever appropriate.

PLEASE NOTE As part of the EMBO Publications transparent editorial process initiative (see http://www.nature.com/msb/journal/v6/n1/full/msb201072.html), Molecular Systems Biology now publishes online a Review Process File with each accepted manuscript. Please be aware that in the

event of acceptance, your cover letter/point-by-point document will be included as part of this file, which will be available to the scientific community. Authors may opt out of the transparent process at any stage prior to publication (contact us at msb@embo.org). More information about this initiative is available in our Instructions to Authors.

If you feel you can satisfactorily deal with these points and those listed by the referees, you may wish to submit a revised version of your manuscript. Please attach a covering letter giving details of the way in which you have handled each of the points raised by the referees. A revised manuscript will be once again subject to review and you probably understand that we can give you no guarantee at this stage that the eventual outcome will be favorable.

Lastly, I would like to acknowledge that this review process took somewhat than is typical for Molecular Systems Biology, in part because August tends to be a difficult month for finding reviewers. I apologize for the delay.

Sincerely,
Editor - Molecular Systems Biology

Referee reports

Reviewer #1 (Remarks to the Author):

This study examined growth of wild-type E. coli (BW25113) and various knockout strains during lambda phage infection. Using the wild-type as the reference, the authors developed a displacement metric to infer the impact of different host factors (involved in sulfur metabolism and programmed ribosomal frame-shifting). This allowed them to propose a putative mechanism (modulation of programmed frameshift) to explain the effects of these host factors. A mathematical model based on this mechanism was able to produce results that were overall consistent with experimental observations.

The work is thoroughly carried out. The quantitative approach of using growth dynamics to infer the underlying mechanism of a signaling network of interest is quite clever. As illustrated, the approach is highly efficient in identifying potential host factors that can contribute to viral infection (positively or negatively); these putative factors can serve as the basis for subsequent, more focused analysis. This overall approach is likely applicable for examining other virus-host interactions, though the authors didn't seem to emphasize this possibility.

Taken together, the work represents a highly significant contribution to the systems biology community. I would recommend its publication in MSB, but the following issues should be addressed/clarified in a revision to strengthen the paper.

Major points:
1. It is surprising that the authors didn't present the growth curves of uninfected knockout mutants. Did they grow similarly as the wild type? If there's significant difference in their growth dynamics (compared to the wild type), shouldn't the difference be corrected in the displacement metric? Judging from the first stage of the infection dynamics (before lysis occurred), it appears that most knockout strains exhibited slower growth, in comparison to the wild type.

2. Is there evidence that miaB and ttcA knockouts don't alter gpG:gpGT ratio significantly?

3. Several points regarding the mathematical models need clarification:

(a) Some rate constants appear inconsistent between the two models. For example, k7 in the first model is one order of magnitude greater than the corresponding rate (k3f) in the alternative model.

Also, the value of k8 is not described (if it is the same as k7, k8 is also one order of magnitude greater than k4f). For fair comparison, the relevant parameters should match between the two models. For this, it would be nice to have a table summarizing the description of parameters and their values so that the readers can compare the two models easily (without it, it is confusing because two models use different notations to denote the same reaction constants (e.g. k7 and k4f)).

(b) The amount of thiolated IscS is increased 100-fold in the alternative model. What is the rationale behind this?

(c) The parameter f (fraction of lytic decision) is set to 1. Is this typo? How can the population dynamics show the recovery after lysis? Also, is the fraction different in different knockout mutants?

(d) How sulfur transfer affects the parameter b (phage burst size) is inconsistent between the two models. In particular, this inconsistency makes b in Fig. 6H and K (IscU and TusA double knockout) artificially different. For the first model (Fig. 6H), b becomes 9.8 (s2U_final=0) whereas for the alternative model it becomes 140 (s2C_final=0).

Because the claim that IscU affects phage replication through competing with TusA for sulfur relies heavily on the modeling analysis (no experimental result is provided in the current paper, though the study by Shi et al suggests competitive binding of IscU and TusA to IscS), the above points question the validity of the modeling analysis to support the claim.

Minor points:

1. On pg. 4, the first line of the second paragraph: what does Figure 2A refer to?
2. On pg. 7 the last line of the second paragraph: there is no Figure 2E.

Reviewer #2 (Remarks to the Author):

This manuscript builds on earlier work by the same group in which they had identified a number of E. coli genes which, when disrupted, interfered with infection by bacteriophage lambda. In the work presented here, they further investigate a subset of these genes, finding a link between tRNA modification and the degree of programmed ribosomal frameshifting (PRF), which in turn influences the efficiency of phage infection. Although the study is carried out entirely with lambda, the work should be of interest to a much wider audience, given the conservation of PRF and the possibility that the enzymes which catalyse the tRNA modifications required for PRF may represent new drug targets.
The authors rely heavily on their newly devised technique for quantitatively analysing the growth kinetics of phage infected cultures, the data for which can be collected in microtitre plate format. This quantitative analysis of phage infection, a vast improvement over old fashioned plaque counting/morphology assays, allows them to incorporate a mathematical model to test their proposed metabolic network connections. By linking a relatively simple competitive binding model of sulphur metabolism into their phage infection/cell growth model, they are able to make and test specific predictions of cell growth, following phage infection of strains deficient in various components of the pathway. The combination of a tractable mathematical model with experimental tests of the predictions which emerge from that model is a key strength of this paper.
The manuscript, including the methods, is written clearly and crisply, with thoughtfully designed figures.
I have just a few minor comments:
One could argue that raw data for all of the biological replicates of the experimental growth curves, rather than just the averaged data, should be shown in Figure 6, (parts C, E,G and particularly I) since it is here that the model predictions are being tested.
I found just one typo, under experimental procedures/assaying lambda PRF, 4th line.
Aliquots..(with?).. arabinose
Table 1 may be unnecessary, as it lists only three of the many viruses which require PRF for efficient infection.

Reviewer #3 (Remarks to the Author):

The authors previously reported that tus proteins are involved in lamda pharge replication. In this paper, they studied detailed mechamism how tus proteins contribute to the viral replication. Tus proteins are sulfur mediators responsible for 2-thiouridine formation at the wobble position of some tRNAs including tRNALys. As lamda pharge gpG has a programmed frameshift signal containing a slippery Lys codon, they speculated that lack of 2-thiouridine in tRNALys might alter the frameshift efficiency of gpG. They measured frameshift activity of gpG in a reporter construct upon knocking out each of tus genes or nmnA. The data clearly demonstrated that lack of 2-thiouridine in any of deletion strains shown above induced frameshift activity of gpG to produce a long form (gpGT), resulting in decreased vival replication. In contrast, it was shown that lack of ISC biogenesis enhanced viral replication. The initial step of sulfur transfer is shared with both systems, ISC biogenesis and 2-thiouridine formation. To be more precise, IscS, cysteine desulfurase, accepts a sunfur atom from cysteine as a persulfide, then transfers it to IscU, a scaffold protein for ISC biogenesis, or to TusA, a mediator for 2-thiouridine formation. As it has been supposed that both IscU and TusA interacts with IscS in competitive manner, lack of IscU might increase the efficiency of 2-thiouridine formation. As expected, the frameshift activity of gpG was completely abolished in the deletion strain of IscU. Simulation of sulfur metabolism in E. coli in silico also supported this idea. Basically, experiments were well executed, and data are convincing. In fact, it is a nice piece of contribution in the field of bacterial regulatory translation as well as more general recoding event for viral expression. But, this reviewer has some concerns to be addressed for publication.

Major comments
If their observation is correct, frequency of 2-thiouridine in tRNALys should be increased in the deletion strain of iscU. They did not show any data of quantifying 2-thiouridine in tRNALys, but just cited Bjork's paper (2004). However, this reviewer could not find any reliable data showing increased s2U formation in the cited paper. To quantify 2-thiouridine in particular tRNA, APM-Northern blotting will be the most convincing technique. In addition, to support the idea, I recommend them to check whether overexpression of TusA also protects frameshifting of gpG.

This study heavily relies on biogenesis of 2-thiouridine mediated by tus proteins. However, they did not cite the key paper in Introduction. It is quite unnatural. The Mol Cell paper by Ikeuchi et al.(2006) should be cited clearly. Also, at the initial step of persulfide sulfur transfer, the competition between IscU and TusA to interact with IscS is the original idea from the above-mentioned paper. Cite this paper properly.

minor comments
In Fig2A, check the color code between infected and uninfected.

1st Revision - authors' response 18 November 2011

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November 15, 2011

Molecular Systems Biology Editors
Nature Publishing Group
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Dear Editors,

We thank you for the reviews you sent last month and are happy to submit with this letter a revised version of our manuscript, "Competing Pathways Control Host Resistance to Virus via tRNA Modification and Programmed Ribosomal Frameshifting", that takes into account all of the reviewers' suggestions. We were encouraged by the reviewers' supportive words and found their advice to be very helpful. I list our responses to the reviewers and corresponding manuscript changes below in the order we received them:

Reviewer 1 Comments

1. The Reviewer thought that we should include uninfected *E. coli* growth curves, and wondered why the infected growth curves were not corrected based on the uninfected growth rates. Based on this comment we have now included all of the uninfected growth curves, to be included in a supplemental file or as "source data". Correcting the infected growth curves was also our own first thought -- in fact, we used this approach in our earlier work (see Maynard et al., *PLoS Genetics* 2010). However, since that time we have tried many methods for classifying the knockout strains based on infection growth curves. After that process, we settled on the metric described in the manuscript. Clustering by uninfected growth rate alone is insufficient to produce the results we describe: as one can see from the uninfected growth curves, most of our strains exhibit a smaller growth rate even while some have a dramatically increased or decreased clearance rate. At the same time, bacterial growth and viral growth are inseparably linked, due to phage replication's dependence on host metabolism, and we found that our attempts to correct for, or eliminate the effect of bacterial growth rate, often distorted the raw data.

2. The Reviewer requested evidence that the miaB and ttcA knockout strains did not exhibit altered gpG:gpGT ratios significantly. This was a good question – to learn the answer, we expressed our gpGT construct in these knockout strains and found that the gpG:gpGT ratio was consistent with wild-type levels. A supplemental figure and the following text were added to the first paragraph on page 13. "We also tested frameshifting frequency for additional strains (*ΔmnmA*, *ΔthiI*, *ΔmiaB*, *ΔttcA*, *ΔhscA*, *ΔhscB*, *Δfdx*, *ΔiscA* and *ΔiscR)* and found frameshifting levels to be consistent with infection dynamics (see Supplementary Figure 1)."

3. The Reviewer had several concerns about the modeling, including the inconsistency of rate constants between models, several parameter values and whether we could justify the comparison of the two models given the structure of each. All of these suggestions were quite valid and we have made substantial changes to the model which strengthen this aspect of the study substantially, even though the final conclusions remain the same. Most importantly, we changed the structure of the equations to determine burst size so that they are consistent with each other. We have also added a table in the Supplement listing each model parameter name and value for both the competitive binding model and the independent effect model. The value of the parameter f was changed to 0.93 for all strains, which is the value we determined from experiments in our earlier work (Maynard et al., *PLoS Genetics* 2010). With respect to the amount of thiolated IscS being increased 100-fold in the alternative model, we added some new text to the Materials and Methods section, pg 27, paragraph 1. "The concentrations of IscU and TusA were set to the same values as in the competitive model. The amount of thiolated IscS, however, was increased 100-fold to prevent it from being limiting." The resulting model and explanation is much better, and we are extremely thankful for these suggestions.

4. The Reviewer mentioned that our conclusions depend heavily on the modeling analysis, and that "no experimental result is provided in the current paper, though the study by Shi et al suggests competitive binding of IscU and TusA to IscS". This was listed to justify the importance of getting the modeling right, which we believe that we have now done (see the above point). However, based on Reviewer 3's suggestions, we have now shown, using an APM Northern Blot, that the hypomodified fraction of tRNALys in the iscU KO is significantly reduced compared to WT levels in BW25113. A new figure and text were added to describe this result, which is an exciting new piece of evidence.

5. A typo: "On pg. 4, the first line of the second paragraph: what does Figure 2A refer to?" We deleted the reference. Thanks!

6. Another typo: "On pg. 7 the last line of the second paragraph: there is no Figure 2E." We changed "Figure 2E" to "Figure 2C". Thanks!

Reviewer 2 Comments

1. The Reviewer requested the full raw data set for the growth curves in Figure 6C,E,G and I. We have added a Data Source file containing the individual replicates.

2. A typo: "under experimental procedures/assaying lambda PRF, 4th line. Aliquots..(with?).. arabinose." We fixed this. Thanks!

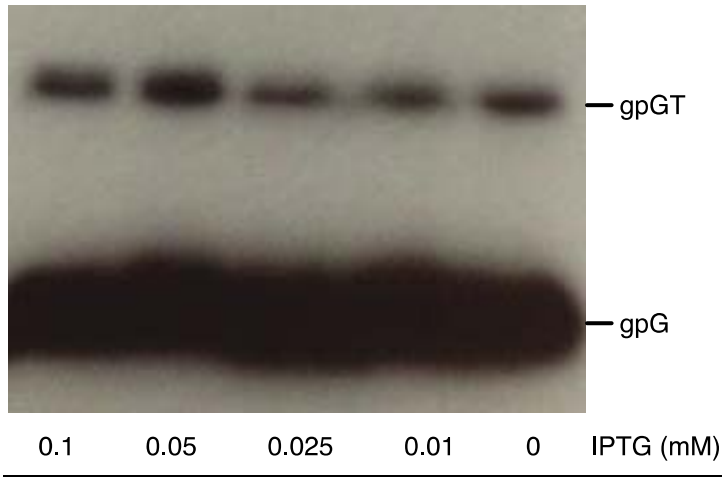
3. The Reviewer suggested that Table 1 may be unnecessary, "as it lists only three of the many viruses which require PRF for efficient infection." We include a reference to other important viruses which utilize PRF, but thought that it might be interesting for readers to get a hint for the conservation we see between our system and human disease.

Reviewer 3 Comments

1. The Reviewer strongly recommended the use of APM Northern Blotting to quantify the s2U modification in our strains. This was a great suggestion! We performed an [(N-Acryloylamino)phenyl] mercuric chloride (APM) Northern blot to determine the fraction of

s2U34 thiolated tRNALys in E. coli BW25113 and iscU KO. We found a small fraction of hypomodified tRNALys in BW25113 and were not able to detect any hypomodified fraction in the iscU KO. This observation agrees what others have shown in *B. subtilis* and with our computational model. We have added new text (Paragraph 2, pg 16) and a new figure (Figure 7, previous Figure 7 is now renamed Figure 8) to reflect this exciting new piece of data.

2. The Reviewer also suggested that we "check whether overexpression of TusA also protects frameshifting of gpG." This was a great suggestion. We created a strain where we could induce *tusA* expression above wild-type levels while monitoring gpGT and gpG expression using our pBAD-lambdaGT plasmid. At levels of *tusA* induction where the cell growth was not significantly altered, we were unable to reproducibly detect a significant change in gpGT levels (see figure below). We also examined infection curves for *tusA* overexpression strains and found little change in the infection dynamics. We find these results very interesting. One explanation, for the weak effect on gpGT levels when tusA is overexpressed could be explained by the higher Kd of TusA, relative to IscU, in binding to IscS. As mentioned in the manuscript, IscU has been shown to be able to displace bound TusA (see Shi et al, 2010). We also found that our computational model supports this view. We have not included these results in the manuscript as they do not alter the main conclusions and we would like to investigate them further.



3. The Reviewer drew our attention to a critical paper that we failed to cite. We apologize for this important oversight. The citation is now contained within the introduction (page 4, paragraph 1) and in the first paragraph on page 10.

4. A typo: "In Fig2A, check the color code between infected and uninfected." We fixed the legend to agree with the text and plot. Thanks!

We thank you and the Reviewers again for your consideration and advice. Please feel free to contact me if you need any further information. I look forward to hearing from you!

Cheers,

Markus Covert

Markus Covert

2nd Editorial Decision 08 December 2011

Thank you again for submitting your work to Molecular Systems Biology. As you will see below, the reviewers are now largely satisfied with the revisions made to this work, and are generally supportive of publication. The first reviewer, however, has some minor suggestions for changes, which we would ask you to address in a final revision of the present work.

In addition, when preparing your revised manuscript, please address the following format and content issues:

We ask that you provide the mathematical models presented in this work in a common machine-readable format as supplementary material. You strongly encourage authors to supply biological models in SMBL format whenever possible, and to submit the models to a public repository like BioModels or JWS Online. The accession number resulting from repository submission should be mentioned in the Methods section of this manuscript.

The current title exceeds our 100 character maximum (including spaces), and we therefore encourage you to select a slightly shorter and simpler title. Perhaps, "Competing metabolic pathways control viral frameshifting and host resistance"?

Please resubmit your revised manuscript online, with a covering letter listing amendments and responses to each point raised by the referees. Please resubmit the paper **within one month** and ideally as soon as possible. If we do not receive the revised manuscript within this time period, the file might be closed and any subsequent resubmission would be treated as a new manuscript. Please use the Manuscript Number (above) in all correspondence.
Thank you for submitting this paper to Molecular Systems Biology.

Sincerely,
Editor - Molecular Systems Biology

Referee reports

Reviewer #1 (Remarks to the Author):

I'm overall satisfied by the revision and responses. And I recommend for its publication in MSB

On point 1, with regard to inclusion of uninfected growth curves for E. coli mutants, I find the authors' arguments plausible. Nonetheless, given that the additional data were only provided as source files (I didn't try to process them), it's difficult to evaluate that point.
I would like to make two recommendations. First, in addition to the source data, I suggest the authors including line graphs for these data and include them as supplemental information.

Second, I suggest the authors to include the clarification in the response letter in the paragraph where they described their metrics.

Reviewer #3 (Remarks to the Author):

The revised version contains an adequate additional experiment (APM Northern) which clearly showed a competition between IscU and TusA to transfer the persulfide sulfur from IscS. Now I recommend this manuscript for publication in MSB.

2nd Revision - authors' response 14 December 2011

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December 12, 2011

Molecular Systems Biology Editors
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USA

Dear Editors,

We thank you for the reviews and comments and are happy to resubmit with this letter a revised version of our manuscript, "Competing metabolic pathways control viral frameshifting and host resistance". I list our responses to the reviewers and corresponding manuscript changes below in the order we received them:

Reviewer 1 Comments

1. The Reviewer thought that in addition to the data source files for the infection curve plots, that we should provide line plots of both uninfected and infected data within the Supplementary Information section. Based on this comment we have now included all of the growth curves in the in a supplemental file and made the following changes to the text:

   In reference to Figure 3, the following has been added to the second paragraph on pg. 8, "see Supplementary Figure 1 for line plots of individual replicates."

   In reference to Figure 4, the following has been added to the first paragraph on pg. 11, "see Supplementary Figure 2 for line plots of individual replicates."

   In reference to Figure 6B-E and Figure 6F, G, the following has been added to the second paragraph on pg. 15, "see Supplementary Figure 4 for line plots of individual replicates."

   In reference to Figure 6I,J, the following has been added to the first paragraph on pg. 16, "see Supplementary Figure 4 for line plots of individual replicates."

2. The Reviewer also requested that further clarify our metric "Host Resistance" within the main text of the paper. We have made the following additions to paragraph 2 on pg. 7, "In earlier work, we found that it was useful to normalize these infection time course experiment vectors by the growth rate (Maynard et al, 2010). However, we have since found that bacterial growth and viral growth are tightly linked, and our attempts to numerically correct for or eliminate the effect of bacterial growth rate often distorted the raw data. Bacterial growth is therefore an important (but not sufficient) consideration in classifying our strains."

We also changed the first sentence of Paragraph 3, pg. 7 to, “As a result, we determined our new metric simply by calculating the Euclidean distance between the knockout and wild-type strain time-course vectors under infection conditions.”

Editor Comments

In addition, per the recommendations of the editors, we have uploaded the source code for our model to simtk.org. Simtk.org was developed by Simbios, the National NIH Center for Biomedical Computing focusing on Physics-based Simulation of Biological Structures. We have added the following sentence to the last paragraph on page 24, “The source code for our model can be downloaded at https://simtk.org/home/lambda-tus.”

Here, we also provide our ‘standfirst text’ and our ‘bullet points’ (see below).

Standfirst Text
Viral infection depends on a complex interplay between host and viral factors. Here, the authors link host susceptibility to viral infection to a network encompassing sulfur metabolism, tRNA modification, competitive binding, and programmed ribosomal frameshifting.

Findings in Bullet Points
(1) the iron-sulfur cluster biosynthesis pathway in Escherichia coli exerts a protective effect during lambda phage infection, while a tRNA thiolation pathway enhances viral infection
(2) $tRNA^{Lys}$ uridine 34 modification inhibits programmed ribosomal frameshifting to influence the ratio of lambda phage proteins gpG and gpGT
(3) the role of the iron-sulfur cluster biosynthesis pathway in infection is indirect, via competitive binding of the shared sulfur donor IscS
(4) Based on the universality of many key components of this network, both in the host and the virus, these findings may have broad relevance to understanding other infections, including viral infection of humans.

We thank you and the Reviewers again for your consideration and advice. Please feel free to contact me if you need any further information. I look forward to hearing from you!

Cheers,

Markus Covert