Author’s response to reviews

Title: Sp110 transcription is induced and required by Anaplasma phagocytophilum for infection of human promyelocytic cells

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Version: 3 Date: 14 August 2007

Author’s response to reviews: see over
August 14, 2007

The Editor

BMC Infectious Diseases

Ref.: Re-Revised manuscript 4498656341416019

Dear Editor,

Thank you for the very helpful Reviewer comments to the revised version of our manuscript. We have considered is suggestions when preparing the re revised manuscript in the following way:

General comments:

1. Comment: Quantitative reverse transcriptase PCR. The authors state that they have used the “comparative Ct method” for establishing mRNA levels. However, they do not reference or further describe the technique, and it appears that this may be incorrect, minimizing the potential importance of their novel observation. The correct formula to use for the Comparative Ct method and to determine fold change in expression level of a gene of interest is the “delta delta Ct” method (see http://dna-9.int-med.uiowa.edu/RealtimePCRdocs/Compar_Anal_Bulletin2.pdf). The advantage of this method is that it will almost certainly accentuate the slope of the curve determined to show fold difference in Sp110 transcription between infected and uninfected cells. The disadvantage is that the calculation results in a single curve that describes fold change. At minimum, the method should be clarified in the text, and if not applied, the delta delta Ct method should be used. Response: The Reviewer is right, the comparative Ct method used in our studies uses the delta delta Ct algorithm. As suggested by the Reviewer, the method used was clarified in the text (Methods section and Figure 1 legend).

2. Also, in figure 1, A. phagocytophilum msp4 transcription is provided as “normalized Ct values”, yet it is unclear how this calculation is done, especially considering that the authors claimed that they used a standard curve for msp4 determination. If so, why isn’t this shown as msp4 copy numbers as in figure 2? Response: As indicated in Methods section, msp4 transcription described in figure 1 was calculated using the delta delta Ct method against beta actin and is therefore provided as normalized Ct values. However, in figure 2 we determined msp4 gene copy numbers against a PCR standard curve. Therefore, both methods and figures are correct as described in the re-revised manuscript.

3. The assessment of viability helps to somewhat clarify one issue, yet raises another. Was viability the same for all transfectants and RNAi knockdowns? This is important since if viability was much higher in the ARP3 RNAi control than in either the Sp110 or PSGL1 knockdowns, the differences in A. phagocytophilum quantities could be more similar. I suspect that this is not the case, but the authors should provide these data for an accurate interpretation. Response: As suggested by the Reviewer, the last sentence of page 7 in Results and Discussion section was
revised to indicate that “Twenty four hours after RNAi, 63±1% cells were viable and cell morphology was not affected in all treatments”

Major Compulsory Revisions:

1. Please describe individually the viability of cells after ARP3, PSGL-1, and Sp110 RNAi transfection. **Response:** See response to comment 3 above.
2. With reference to figure 1, please specify whether the "comparative Ct method" refers to the DDCt (delta delta Ct) method that is most appropriate for accurate transcriptional fold change quantification. Also, please specify whether the quantification of msp4 levels shown in figure 1 was done by "comparative Ct method" or by standard curve as described in the methods. **Response:** Please see response to comment 2 above. Also, as suggested by the Reviewer, it was clarified in figure 1 legend that the delta delta Ct method was used to measure msp4 mRNA levels in this experiment.

Thank you for considering our manuscript for publication in BMC Infectious Diseases. Looking forward to hearing from you soon.

Sincerely yours,

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