Reviewer's report

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

Version: 2 Date: 6 August 2007

Reviewer: J. Stephen Dumler

Reviewer's report:

General
The authors provided a revised version where they describe the use of RNAi and qRT-PCR to study the relevance of Sp110 during Anaplasma phagocytophilum infection of granulocytes. This version is improved over the prior submission, and provides interesting and important new information. I am slightly disappointed that the new version did not expand the data present in the first submission, but none-the-less, the data appear solid. There are still several small problems that should be addressed and resulted from the author revisions, as cited below. I'm sorry to request additional changes, but I would like this manuscript to be very accurate since it describes a new finding that could be cited repetitively.

General comments:
1. 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.

Also, in figure 2, 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?

2. 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.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Please describe individually the viability of cells after ARP3, PSGL-1, and Sp110 RNAi transfection.

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.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

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What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.