Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: WJBC Review 7174-edited.doc).

Title: Thioredoxin and glutaredoxin-mediated redox regulation of ribonucleotide reductase

Author: Rajib Sengupta and Arne Holmgren

Name of Journal: World Journal of Biological Chemistry

ESPS Manuscript NO: 7174

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Comments of Reviewer 1 (00227723):

The authors provide a useful short review on redox regulation of ribonucleotide reductase. The review is informative for the reader who needs an introduction to this field. Better linking of the text to the figures would improve the final version. This is particularly true for Figure 2, in which many amino acids are indicated. The text does not describe the roles of these amino acids in detail. Addition of a few sentences to give this detail would help the readability of the review. The text is generally written clearly. There are a few required language corrections: Page 4: the the abstraction → the abstraction Page 5: the storage of radical → storage of the radical Page 6: Several evidences support → Several findings support Page 7 it has been also shown → it has also been shown Page 8: there exist a → there is a Page 8: In presence → In the presence Page 8: that the mammalian cells have have a → that mammalian cells have a Page 9: mothiol → monothiol Page 9: It suggests → This suggests Page 9: a study with mouse hepatocytes, suggested → a study in mouse hepatocytes suggested Page 10: in resting state → in resting state Page 10: the role of the thioredoxin system (or DTT) was suggested to facilitate the recognition of the network and allowing → the suggested role of the thioredoxin system (or DTT) was to facilitate recognition of the network and allow

Reply:

We would like to thank the Reviewer for the useful comments that helped us to improve our manuscript. The manuscript has been modified according to the suggestions proposed by the reviewer.

We have modified the figure legends of Figure 2 with the description of the roles of the amino acids.

Page 4: We've corrected "the the abstraction" to "the abstraction".
Page 5: We've changed "the storage of radical" to "storage of the radical".
Page 6: We've changed "Several evidences support" to "Several findings support".
Page 7: We've corrected "it has been also shown" to "it has also been shown".

Page 8: We've changed "there exist a" to "there is a".
Page 8: We've changed "In presence" to "In the presence".
Page 8: We've corrected "that the mammalian cells have have a" to "that mammalian cells have a".
Page 9: We've corrected "mothiol to "monothiol".
Page 9: We've changed "It suggests" to "This suggests".
Page 9: We've changed "a study with mouse hepatocytes, suggested" to "a study in mouse hepatocytes suggested".
Page 10: We've changed "in resting state" to "in resting state".
Page 10: We've changed "the role of the thioredoxin system (or DTT) was suggested to facilitate the recognition of the network and allowing" to "the suggested role of the thioredoxin system (or DTT) was to facilitate recognition of the network and allow".

(2) Comments of Reviewer 2 (00289666):

In general, the authors have presented a nice, concise review of ribonucleotide reductase regulation, but there are a few issues that need to be addressed before the manuscript can be accepted for publication. First, the mechanism of RNR will be unfamiliar to the non-expert and thus a diagram of the mechanism should be the first figure in the review. Second, when discussing important residues in the R1 and R2 subunits (second paragraph of the "Classification and catalysis" section), no residue number are cited. This makes it difficult for the reader to reconcile the content of this paragraph with Figure 2. The authors should add these details to the text. In the section describing the role of Trx and Grx as electron dodnors, there are also a few problems. First, as a minor point, kcat should be lowercase, not uppercase. Second, the text refers to kcat/Km value, whereas the figure referenced show only the moles of product produced as a function of the different redox systems employed. Moreover, the trend cited in the text is not reflected in the figure. This leads to a very confusing presentation. Finally, the figure legends and figures need some work. In general, any reader should be able to look at the figures and figure legends and get a good sense of the paper. Figure 1 in the manuscript has only a minimal legend despite significant content. The authors should describe the ATP cone, which is circled, and its significance. There is a cofactor present shown in a CPK-like representation. This should be described. The color coding of the subunits themselves should be briefly noted. Finally, the magenta and reddish-brown subunits are too similar in color. Perhaps making one of them orange would be better. In figure 2, once again the legend is minimal. Important residues for RNR function are shown, but there roles are not described at all. At a minimum, the residues that form radicals should be highlighted in both the Figure and its legend. Moreover, these residues should be described in the text, along with citing the appropriate references describing their roles. The legend for figure 3 is fine, but the figure itself only shows redox on the left-hand subunit. The right hand subunit (I assume) undergoes the same process, but it is only partially depicted. If this was omitted for claerity, it should be noted in the legend.

Reply:

We would like to thank the reviewer for his careful reading and helpful comments. We have taken into account all suggestions and addressed the raised issues trying to provide necessary clarifications and improvements. The manuscript has been modified according to the suggestions proposed by the reviewer.

Firstly, we have inserted a new figure (Figure 1B) showing the proposed reaction

mechanism of the RNR catalysis. Secondly, the text and the figure legends of Figure 2 have been modified with the description of the roles of the amino acids. Thirdly, we have changed the "kcat" into lowercase.

We have inserted a Table describing the Kinetic parameters (v_{max} and k_m) of Trx1, Grx1, Grx2, and Grx2C40S for reduction of mouse RNR complex. This will clearly describe the comparative profile of different redox systems.

The legend of Figure 1A has been modified by describing the significance of ATP cone and to describe the presence of substrate in R1 subunit and dinuclear iron center in R2 subunit.

The figure 1A has been adopted from Logan et. al. (Ref: 9) and thus the original colour have been retained. This is based on the crystal structures of the R1 and R2 proteins (PDB 1RLR and 1RIB). The figure legend has been modified to describe that.

We have inserted a new figure (Figure 1B) describing the proposed reaction mechanism of RNR catalysis and the legend of the Figure 2 has been modified. Both of the figures describe the functions of different amino acids residues for RNR catalysis. The function of the thiyl radical (Cys 439) has also been shown in Figure 1B. Moreover, the text has been modified to describe the functions of different residues.

For simplicity, only the reduction of active site of one subunit by the C-terminal shuttle dithiols of the neighboring subunit is shown in Figure 3. We have modified the legend to describe that.

(3) Comments of Reviewed 3 (02445169):

This review article is written by experts in the field of thiol reductase. The prospect of targeting TR and GR to interfere with RNR catalysis also has great therapeutic value. However, a main concern of this review article is the lack of sufficient new information. In 2010, the same authors published a review article (Free Radical Biology & Medicine 49 (2010) 1617: 1628) that covered almost all key points of this submission, and the figures in this submission are either replicated or derived from the figures in the 2010 review article. The authors do mentioned some recent data, such as the 2012 Holmgren JBC paper about the ability of glutathion/glutaredoxin to reduce thioredoxin 1, but such new information is too little. The authors also included a paragraph discussing the role of thioredoxin system in the activity of class 3 RNR. However, such discoveries have been made more than 10 years ago. In summary, I feel that this submission in its current state does not contain sufficient new information to merit publication at World Journal of Biological Chemistry.

Reply:

We would like to thank the Reviewer for the comments that helped us to improve our manuscript. The manuscript has been modified according to the suggestions proposed by the reviewer.

The present review focuses on the role of thioredoxin and glutaredoxin systems in the redox reactions of the RNR catalysis. We have incorporated all the new information related to the topic. In 2009, a study by Zahedi Avval et. al. characterized the molecular mechanisms of thioredoxin and glutaredoxin as hydrogen donors for mammalian s

phase ribonucleotide reductase (Ref 26: J Biol Chem 2009; 284: 8233-8240). This has been discussed in the present review. Recently, Gustafsson et. al. (Ref 31: J Biol Chem 2012; 287: 39686-39697) have characterized the Trx1 system as the physiologically relevant electron donor for RNR in Bacillus anthracis. We have also discussed that in the present review.

In the present review, we have only adopted Figure 1B from Holmgren et. al. (Ref: 4). However, Figure 1A has been adapted from Logan et. al. (Ref: 9), Figure 2 and 3 have been adopted and modified from Holmgren et. al. (Ref: 4), Figure 4 and Table 1 have been adapted from Zahedi Avval et. al. (Ref: 26).

In 2010, we have published a review article (Free Radical Biology & Medicine 49 (2010) 1617: 1628) on the use of thiols by Ribonucleotide Reductase. Therefore, in the present review, we have discussed the role of thioredoxin and glutaredoxin systems in the redox reactions of the RNR catalysis in addition to the many recent studies (such as Ref 17: Biochem J 2011; 433: 303-311, Ref 21: J Cell Sci 2010; 123: 2402-2412, Ref 22: Cancer Res 2010; 70: 9505-9514, Ref 23: J Biol Chem 2012; 287: 38210-38219, Ref 37: Plant J 2010; 64: 825-838, Ref 46: Mutagenesis 2013; 28: 653-660, Ref 47: J Biol Chem 2012; 287: 35768-35778, Ref 52: Int J Gynecol Cancer 2013; 23: 659-666, Ref 53: Cancer Chemother Pharmacol 2011; 68: 193-205, Ref 54: J Pept Sci 2011; 17: 756-762).

3 References and typesetting were corrected

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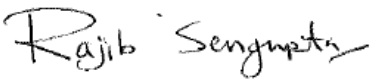

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