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Interactive comment on "The nonenzymatic template-directed ligation of oligonucleotides" *by* A. V. Lutay et al.

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The comment of Anonymous referee 2 concerns the validity of our suggestion about the possible role of 2′,5′-phospodiester bonds in early RNA world. Having the nice opportunity to give the Author Comments, we would like to recreate the line of our arguments in order to explicate our statements. It is evident that the first oligomers of RNA-like molecules synthesized in prebiotic condensation reactions comprehended many of possible isomeric bonds (as to the phosphodiester bonds, both 2′,5′- and 3′,5′- were included). This is in accordance with the variety of experiments simulating possible prebiotic reactions (Ferris, 1993; Kanaya et al., 1986; Orgel et al., 1974; Sawai, 1980; Usher et al., 1976). In our paper we describe the template-directed ligation reaction of 2′,3′-cyclic phosphate resulting in the preferred synthesis of 2′,5′-phosphodiester bonds. RNA fragments bearing 2′,3′-cyclic phosphates are produced in the transesterification reaction both of 2′,5′- and of 3′,5′- ribolinkages, that proceeds in the presence of metal ions under the broad range of conditions. Thus, the existence

of 2′,3′-cyclic phosphates in prebiotic soup is without doubt. The sequence of the ligation products, originated from the two oligonucleotides A and B, seems to be a novel one, that contains both A and B sequences (see the Scheme 1)

..$A_{n-1}A_n$>p + B_1B_2.. -> ..$A_{n-1}A_nB_1B_2$..

(Scheme 1)

It was shown that oligoribonucleotides containing both 2′,5′- and 3′,5′- linkages could serve as template or primer in the templated-directed synthesis of RNA (Sawai et al., 2006) (we mentioned that in the Discussion, 4.3). Whatever the mechanism of primitive replication, it was hardly strict too much in respect to 2′,5′/3′,5′ composition of the template. It should have been mentioned that the most to-date believed participants of polymerization reactions are phosphorimidazolides, not 2′,3′-cyclic phosphates or 5′-triphosphates (Huang et al., 2003). Consequently, the novel sequence synthesized in the ligation reaction could be replicated further, resulting in the enrichment of RNA-world diversity. Based on the above, it is reasonable to conclude that such ligation reactions could contribute to the formation of new sequences in early RNA world, whereas the replication could proceed via the different mechanism.

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