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New parameters for accurate prediction of RNA/DNA hybrid duplex stability and their advantage in CRISPR-Cas9 technique

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ABSTRACT

Prediction of thermodynamic stability for RNA/DNA hybrid duplex under a physiological condition is essential to better insight biological reactions and improve gene modifying techniques related to hybrid duplex formation. Previous parameters for predicting hybrid duplex stability were derived in much higher cation concentration (~10 times) compared to the physiological cation concentration. Therefore, new parameters were determined that predict the stability in a physiological condition with significant accuracy. Moreover, the use of new parameters to design efficient guide RNA for CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9 technique was demonstrated.

INTRODUCTION

RNA/DNA hybrid duplex forms not only as the intermediate in cellular reactions like replication, transcription, reverse transcription, but also in therapeutic techniques, for example, gene editing CRISPR-Cas9 technique.[1] *In vivo* development of the technique requires precise prediction of hybrid duplex stability in the physiological condition. Duplex stability can be predicted well using nearest-neighbor (NN) model.[2] According to the model, thermodynamic stability of duplex can be determined by the summation of the free energy changes due to the formation of all the NN base pairs and the helix initiation. Based on the model, NN parameters for hybrid duplex were developed that can predict well the duplex stability in 1 M NaCl solution.[3] The salt concentration, however, is almost 10 times higher than the physiological salt concentration. Since cation concentrations affect duplex stability largely, the previous parameters may not be useful under an intracellular salt condition. Here, we investigated the applicability of previous parameters, and therefore improved new NN parameters in the solution closer to physiological environment for the development of the gene editing technique.

RESULTS AND DISCUSSION

Intracellular environment comprises monovalent (Na^+, K^+) and divalent cations (Mg^{2+}, Ca^{2+}).[4] To mimic the environment, we prepared a cellular cation condition containing 120 mM KCl, 10 mM NaCl,

1 mM $MgCl_2$, 0.2 μM $CaCl_2$, and 10 mM K_2HPO_4 (pH 7.0). The melting curves of hybrid duplex in the cellular cation condition was compared with the melting curves in the buffer solutions containing 1 M NaCl and 100 mM NaCl. The comparison revealed that the free energy change for duplex formation in the cellular condition at 37 °C ($\Delta G°_{37}$) shows close proximity with the $\Delta G°_{37}$ in 100 mM NaCl solution although $\Delta G°_{37}$ in 1 M NaCl solution was quite different from its value under physiological conditions. Next, we verified the validation of the NN model for the hybrid duplex in 100 mM NaCl solution. Then, based on the thermodynamic parameters measured for 38 hybrid duplexes in the 100 mM NaCl solution, new NN parameters were derived. The parameters predicted well the $\Delta G°_{37}$ of the 38 hybrid duplexes with an average prediction error of only 2.9 % and also help to improve designing of single guide RNA (sgRNA) in CRISPR-Cas9 technique. Length shortening of sgRNA helps to decrease the off-target binding and increase the efficiency of the drug.[5] The length of sgRNA should be optimal so that it can form hybrid duplex with target site of optimal stability. Figure 1 showed the advantage of our parameters over the previous parameters in the prediction of hybrid duplex stability in CRISPR technique using a typical example.

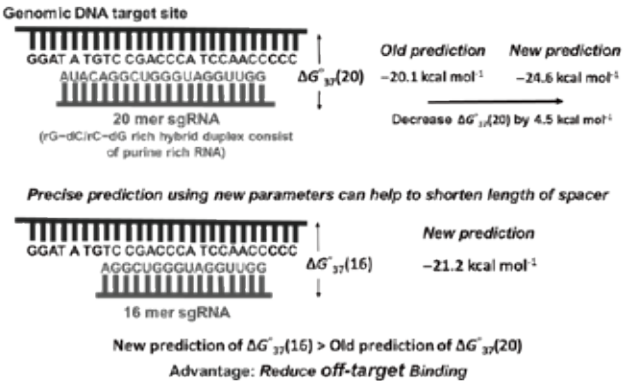

Figure 1. Schematic presentation about the advantage of the precise prediction for hybrid duplex stability in CRISPR-Cas9 technique.

CONCLUSION

The validity of NN model was verified and new NN parameters were determined for hybrid duplex in 100 mM NaCl solution. New parameters predicted well the stability of hybrid duplex in a physiological salt condition. In the improvement of CRISPR technique, the application of our new parameters was also demonstrated.

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