Determinants of siRNA Functional Asymmetry
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Abstract

The use of biological molecules as therapeutics (i.e., biologics) is a rapidly expanding area of pharmaceutical research because of their potential for high specificity and low toxicity. Biologics often utilize native cellular mechanisms to amplify their activity while producing minimal perturbation of cellular function. Short interfering RNAs (siRNAs) are a class of biologics that silence targeted mRNAs via the native eukaryotic regulatory pathway called RNA interference (RNAi). Canonical siRNAs are double-stranded with a central base and a nucleotide 3' overhangs. The RNAi pathway recognizes the siRNA structure and then removes one of the RNA strands to form an active complex called RISC, which finds its target through the now free base pairs of the remaining siRNA strand.

While the recognition of the siRNA by the RNAi pathway is largely structurally based, siRNA activity can vary greatly with siRNA sequence. Our previous work has identified that the 5' terminal nucleotide (TN) and the relative terminal hybridization stability (ΔΔG) of the siRNA are independent predictors of siRNA activity. Here we show that both RISC loading and RNAi activity are influenced by TN and ΔΔG with the strongest influence on functional asymmetry coming from TN. That said, they are not perfect predictors, indicating that other as yet unidentified factors also influence siRNA activity and functional asymmetry.

Conclusions

siRNA mediated knockdown of PKR is primarily controlled by TN sequence and secondarily controlled by ΔΔG.

The TN and ΔΔG predicted siRNA functional asymmetry with the strongest predictions coming from our algorithm ($p < 0.0001$) for the cell-based assay.

Strand loading and initial rate measures of functional asymmetry show less agreement with our algorithm, indicating that other cellular mechanisms may directly influence siRNA loading or RISC activity.

Total loading of siRNAs does not correlate with TN or ΔΔG.

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References


