Using Unlocked Nucleic Acid (UNA) Substitutions to Alter Strand Selection by the RNA Induced Silencing Complex. "Making a Bad siRNA Good."

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Objectives

In some applications, choice of siRNA sequence is constrained and no potent siRNAs are identified. Our objective was to determine if strategic UNA placement can be used to convert a non-functional siRNA into a functional siRNA.

Abstract

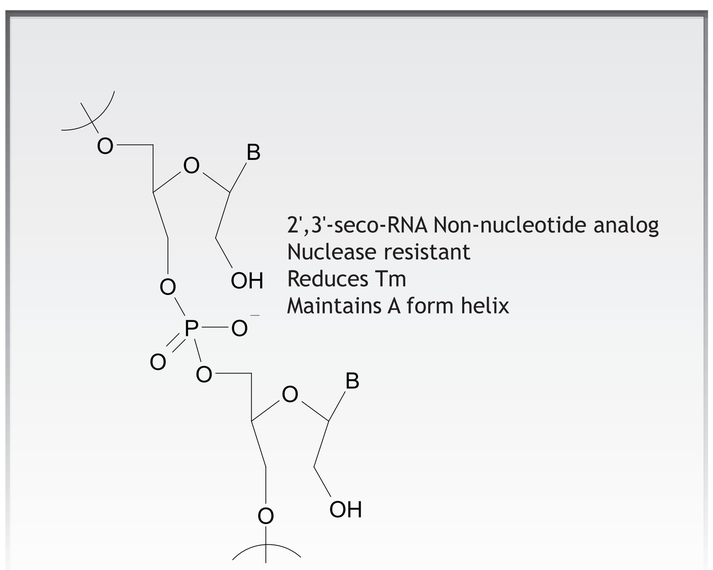
Entry of the desired (antisense) strand into the RNA Induced Silencing Complex (RISC) is a critical determinant of small interfering RNA (siRNA) potency and specificity. For most synthetic siRNA duplexes, both strands enter RISC to some degree. Undesired RISC sense strand entry can cause off-target silencing of genes with sense strand homology. Off-target effects can result from strand cleavage or microRNA-like interactions between the seed region of the incorporated strand and seed matches in off-target mRNAs. Here, we utilized Unlocked Nucleic Acid (UNA) substitutions to alter strand selection to favor incorporation of the desired antisense strand. UNAs are non-nucleotide analogs in which the C2'-C3' bond of the ribose ring is absent. Previous studies have shown that strategic UNA substitutions at the 5' end of the passenger strand and at position seven of the guide strand can reduce off-target effects by >90% (Vaish et al. NAR (2011) 39, 1823).

Previously, we conducted a detailed examination of the IC50 for the antisense and sense strands of a series of siRNAs targeting hnRNPH (Sakurai et al. NAR (2010) 39, 1510). We found that shifting an siRNA by a single nucleotide dramatically altered the relative potency of the antisense and sense strand as measured by luciferase target reporters. Here, we tested whether UNA substitution could "make a bad siRNA good." As a test case, we chose hnRNPH siRNA H5, for which strand selection is highly biased toward undesired sense strand incorporation (IC_{50} of 72 pM for the sense strand and 9.8 pM for the antisense strand). 5' UNA substitution of the sense strand greatly reduced sense strand silencing while increasing on-target silencing by the antisense strand; in effect, converting a non-functional siRNA to a functional one. Thus, UNA substitution can be used to drive incorporation of the antisense strand into RISC for improved on-target silencing and presumably reduced off-target effects by the sense strand. When targeting highly conserved viral regions or single nucleotide polymorphisms, the potential sequence space for siRNA selection is highly constrained. In these cases, UNA modification may be a useful tool to expand the selection of potential siRNA sequences.

The antisense strand can also mediate off-target effects through miRNA-like interactions between the seed region of the antisense strand and seed matches in off-target mRNAs. Several reports have suggested that UNA modification of antisense position seven could reduce seed based off-target effects (NAR (2010) 38, 5761). In one report, combination of a UNA substitution at antisense position seven with 5' UNA sense strand modification, dramatically reduced off-target effects (NAR (2011) 39, 1823). Using antisense and sense strand reporters, we confirmed that UNA modifications at these positions did not compromise the activity of an ApoB siRNA.

Figure 1

Unlocked Nucleic Acid Structure



Nomenclature

Antisense Strand (AS): Complementary to target mRNA. This is the desired strand in RISC.
Sense Strand (SS): Cannot mediate on-target silencing. Can cause toxicity by silencing off-target genes.
UNA: Abbreviated as "U."

Figure 2

Off-Target Effects Due to Incorporation of the Wrong Strand into RISC

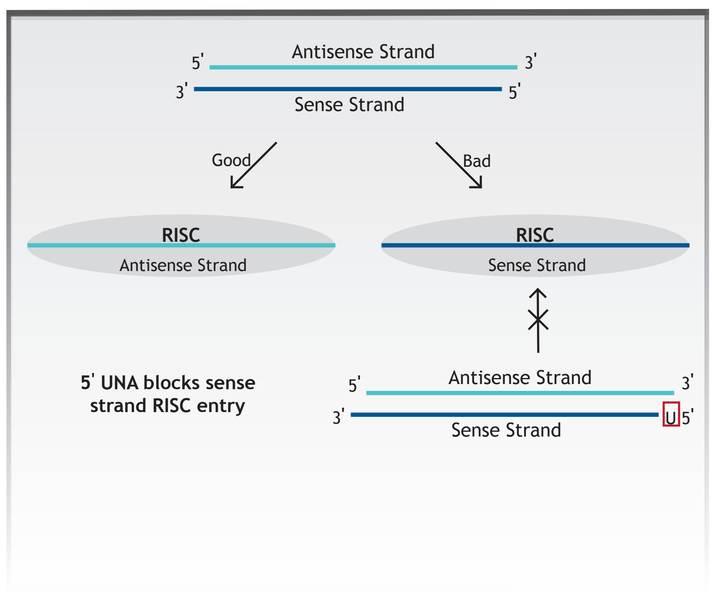


Figure 3

Off-Target Effects Due to Seed Interaction With Off-target Genes - miRNA Mechanism

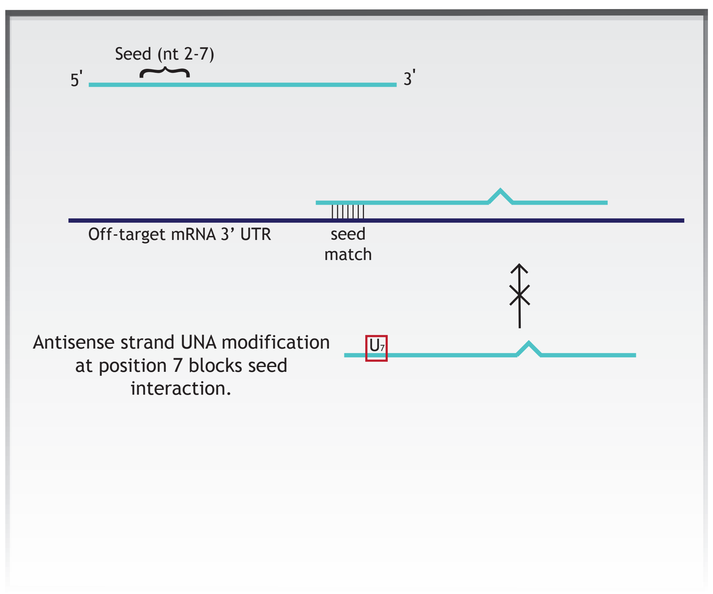


Figure 4

Preferred UNA Modification Format

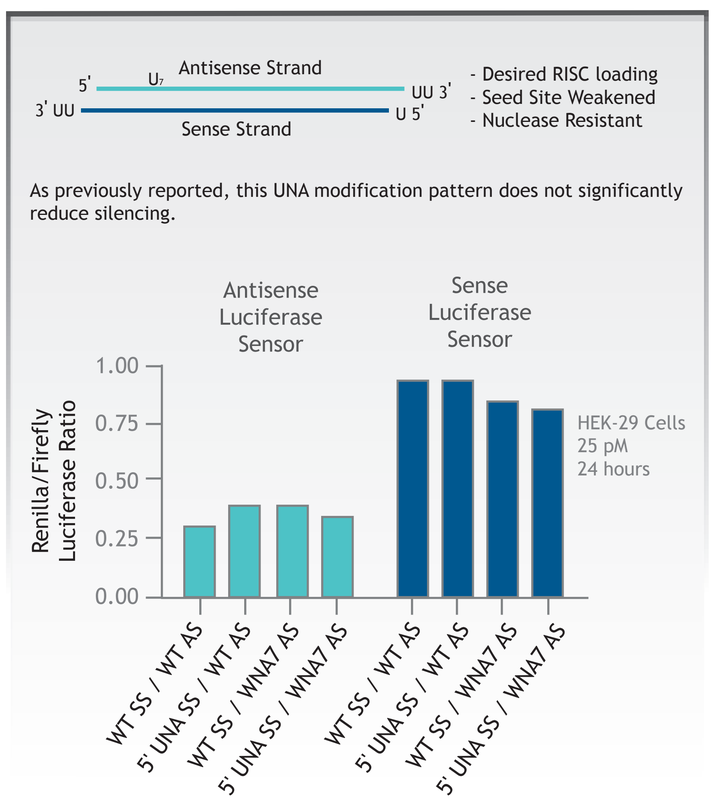


Figure 5

Making a Bad siRNA Good

In a gene walk, we previously identified a "bad" siRNA (hnRNPH H5) where the undesired sense strand is incorporated more efficiently into RISC than the desired antisense strand.
Sense IC_{50} = 9.8 pm Antisense IC_{50} = 72 pm

UNA at 5' position of sense strand reduces sense strand off-target silencing and promotes on-target antisense strand silencing.

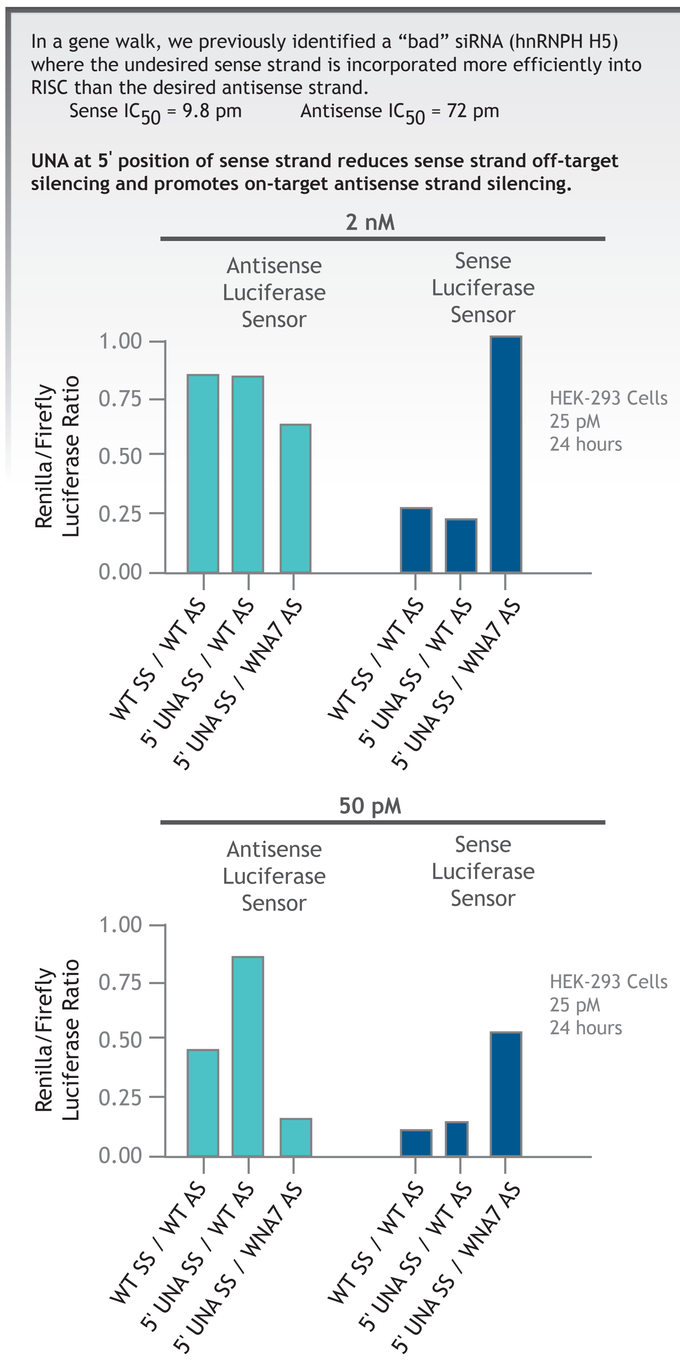
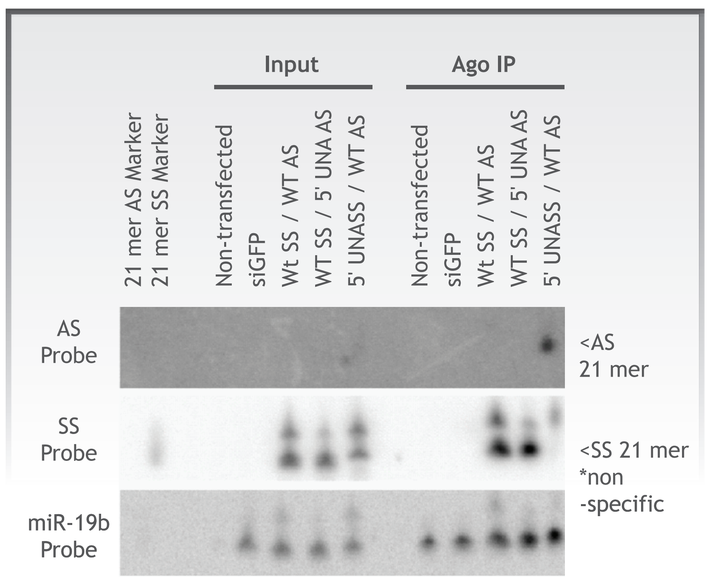


Figure 6

Argonaute Pull-Down Demonstrates That 5' Modification of a Strand Excludes it from RISC.



Conclusion

- UNA modification of 5' end of the sense strand prevents incorporation of this strand into RISC. This reduces the possibility of off-target effects.
- This strategy can be used to make a "bad" siRNA "good."
- UNA modification of position 7 has been reported to reduce seed mediated off-target effects.
- The combination of a 5' sense strand UNA modification and a 7 position antisense strand UNA modifications does not reduce silencing efficacy.

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