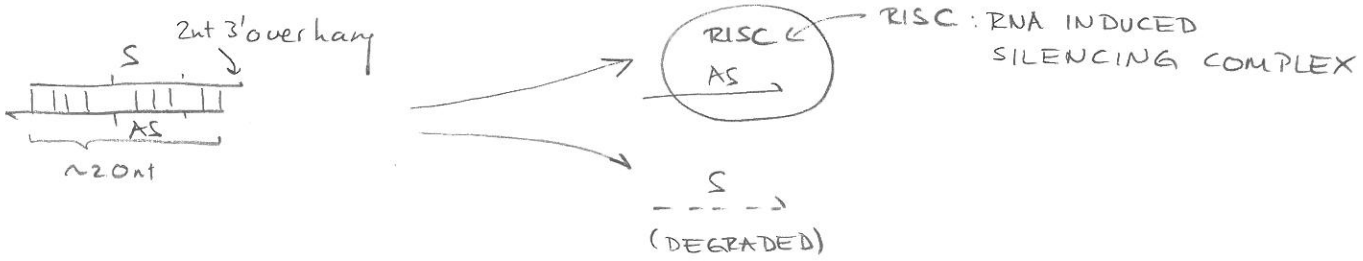
10/27/10

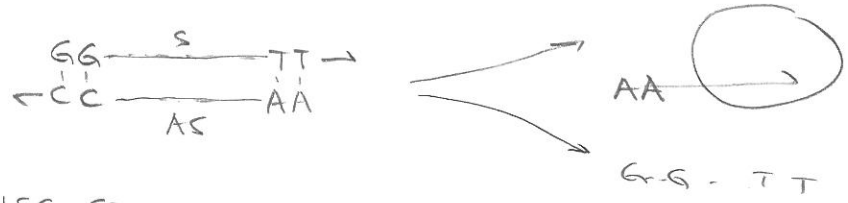
FORMATION OF MATURE MIRNA:



RISC IS A MULTIPROTEIN COMPEX

AN ARGONAUTE PROTEIN (AGO1/2/3 OR 4) IS THE MAIN COMPONENT OF RISC.

STRAND SELECTION (EXAMPLE):



RISC SELECTS THE STRAND WITH THE MORE WEAKLY BOUND 5'END.

MICRORNA TARGETING MECHANISM

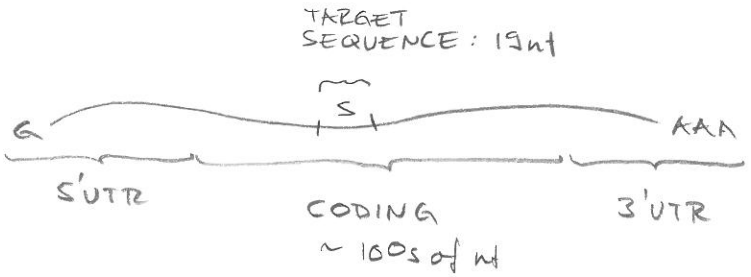
* TARGETING OCCURS IN 5'UTR AND REQUIRES PARTIAL COMPLEMENTARITY
* SEED REGION (NUCLEOTIDES 2-7) IS MOST IMPORTANT FOR TARGET BINDING.
* MOST MIRNA HAVE MANY TARGETS
* MIRNA BINDING CAN LEAD TO TRANSLATION INHIBITION (W/O mRNA DEG.), mRNA DEGRADATION (MOST COMMON) OR OTHER EFFECTS.

MIRNA ROLES:

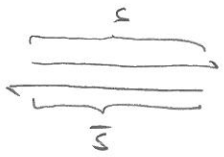
* MIRNA PROVIDE TISSUE IDENTITY AND ARE IMPORTANT IN DEVELOPMENT
  (mir-124 IS ONLY EXPRESSED IN BRAIN, mir-122 IN LIVER ETC.)
* MIRNA CAN BE ONCOGENES (e.g. mir-21) OR TUMOR SUPPRESSORS
* miRNA CAN BE DISEASE MARKERS (SEE E.G. WORK BY M. TEWARI)
* miRNA CAN BE THERAPEUTIC MOLECULES + TARGETS
  (E.G. MIR-26 RESTORATION, J.T. MENDELL CELL 2009)

RNA INTERFERENCE AS A TECHNOLOGY

① TARGET SELECTION



② PREPARE RNA DUPLEX



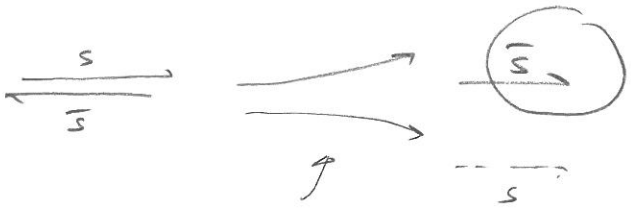
19nt DUPLEX + 2nt OVERHANGS
(PERFECT DUPLEX V/O MISMATCHES)

③ DELIVERY

FOR CELL CULTURE USE POSITIVELY CHARGED LIPIDS (EG. RNAiMAX)

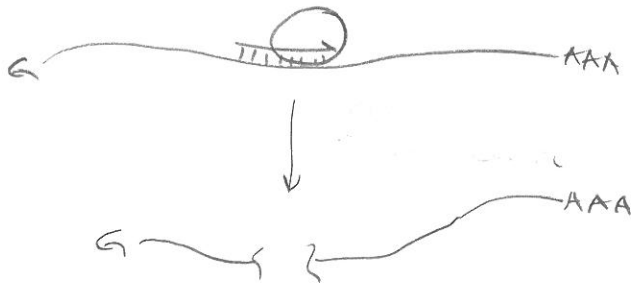
COMPLEX OF NEGATIVELY CHARGED RNA + POS. LIPIDS IS TAKEN UP BY CELLS.

④ MATURE siRNA-RISC FORMATION



DESIGN DUPLEX SUCH THAT CORRECT STRAND IS SELECTED.

⑤ TARGET CLEAVAGE



* CLEAVAGE REQUIRES FULL COMPLEMENTARITY
* ONLY AG2 (NOT AG01/3/4) IS AN ENDONUCLEASE
* CLEAVAGE OCCURS AT THE POSITION OPPOSITE POS. 10 AND 11 FROM 5'END OF siRNA
* siRNA/RISC ACTS CATALYTICALLY