

Biomolecular Engineering and Applications of Nucleic Acids

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Brief Personal Background

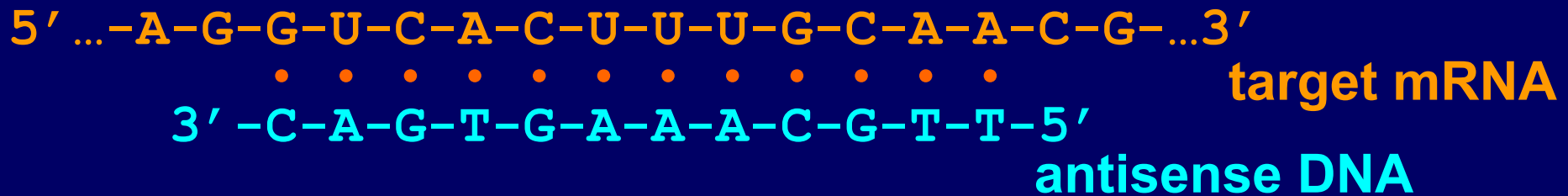
- B.ChE – Georgia Tech – 1994
 - Cardiovascular Fluid Mechanics Laboratory
- MS in ChemE Practice – MIT – 2000
 - At Merck in PA (vaccines) and Dow in TX
- Sc.D. in ChemE – MIT – 2002
 - Co-Advised in Mass. General Hospital
- Post-Doc – Stanford Genome Technology Center – 2002-3
 - Department of Biochem., School of Medicine

Biomolecular and Biotechnology Drugs

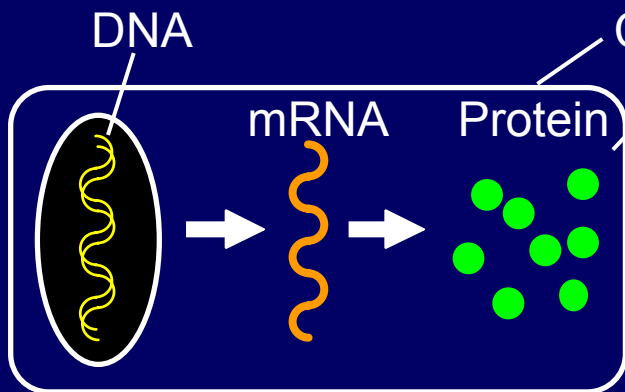
- More than 250 million people have already benefited
- 371 biotechnology medicines in development for nearly 200 diseases
- 95 biotechnology medicines already approved and available to patients
- Most early medicines were protein drugs
 - Recombinant insulin, HGH, Clotting factor, and EPO
- First antisense drug approved in 1998

Principle of Antisense Activity

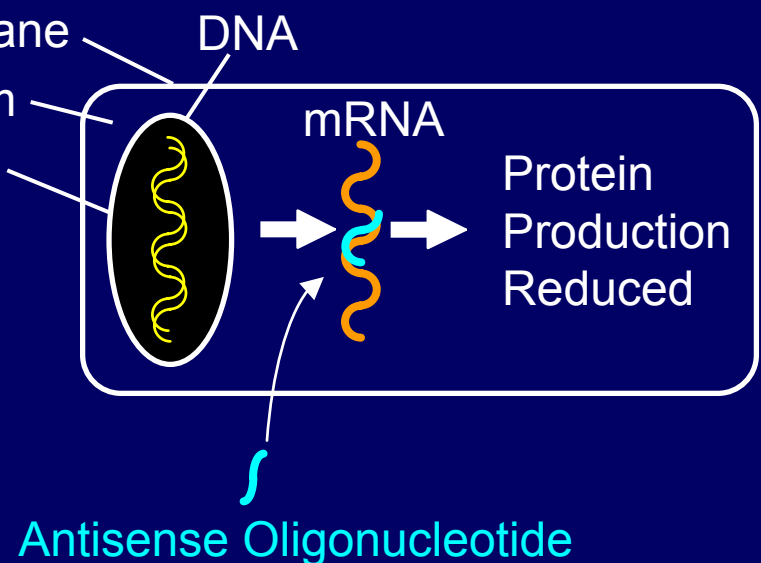
Inhibition by complementary base pairing



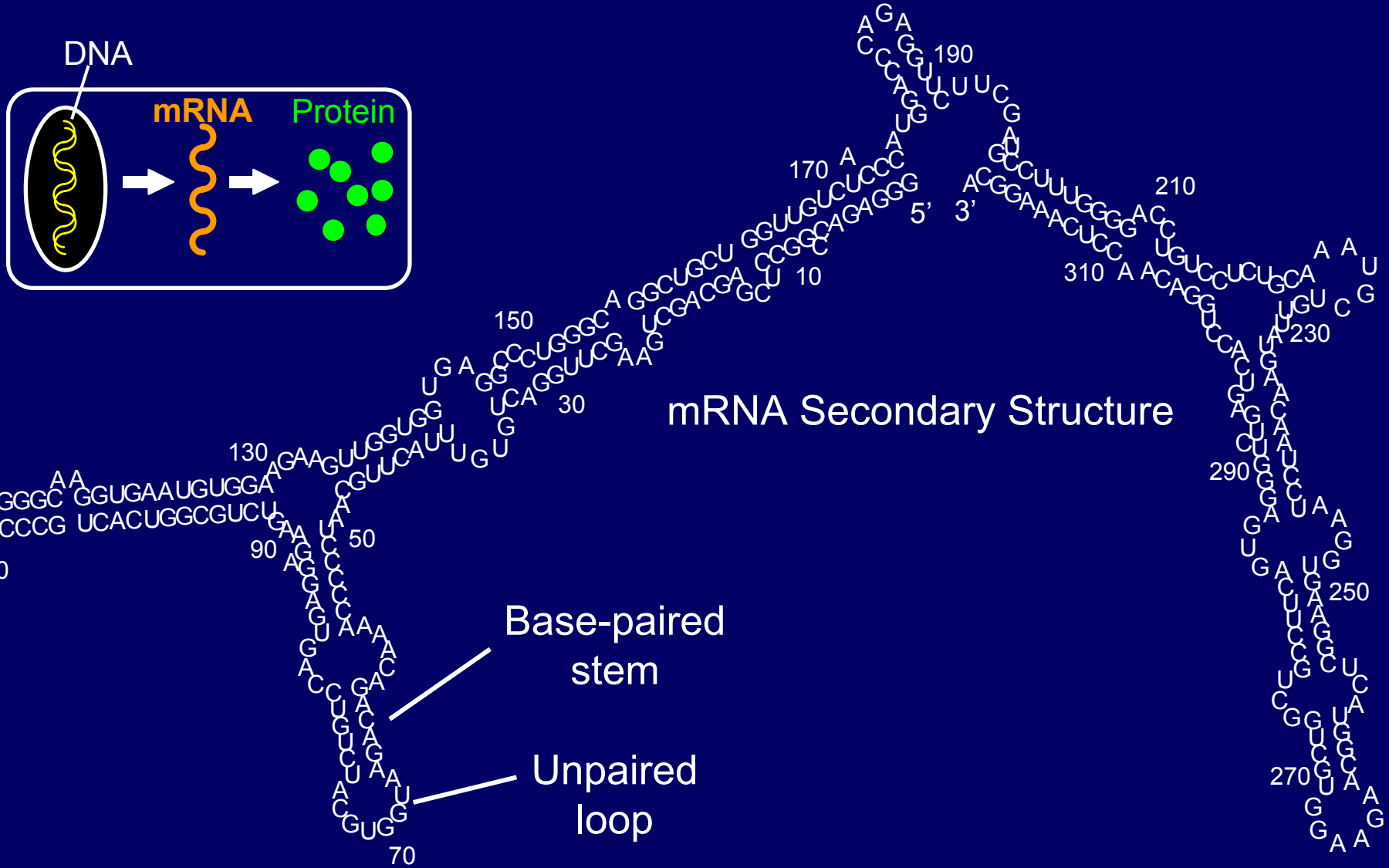
Normal Protein Production



Antisense Inhibition

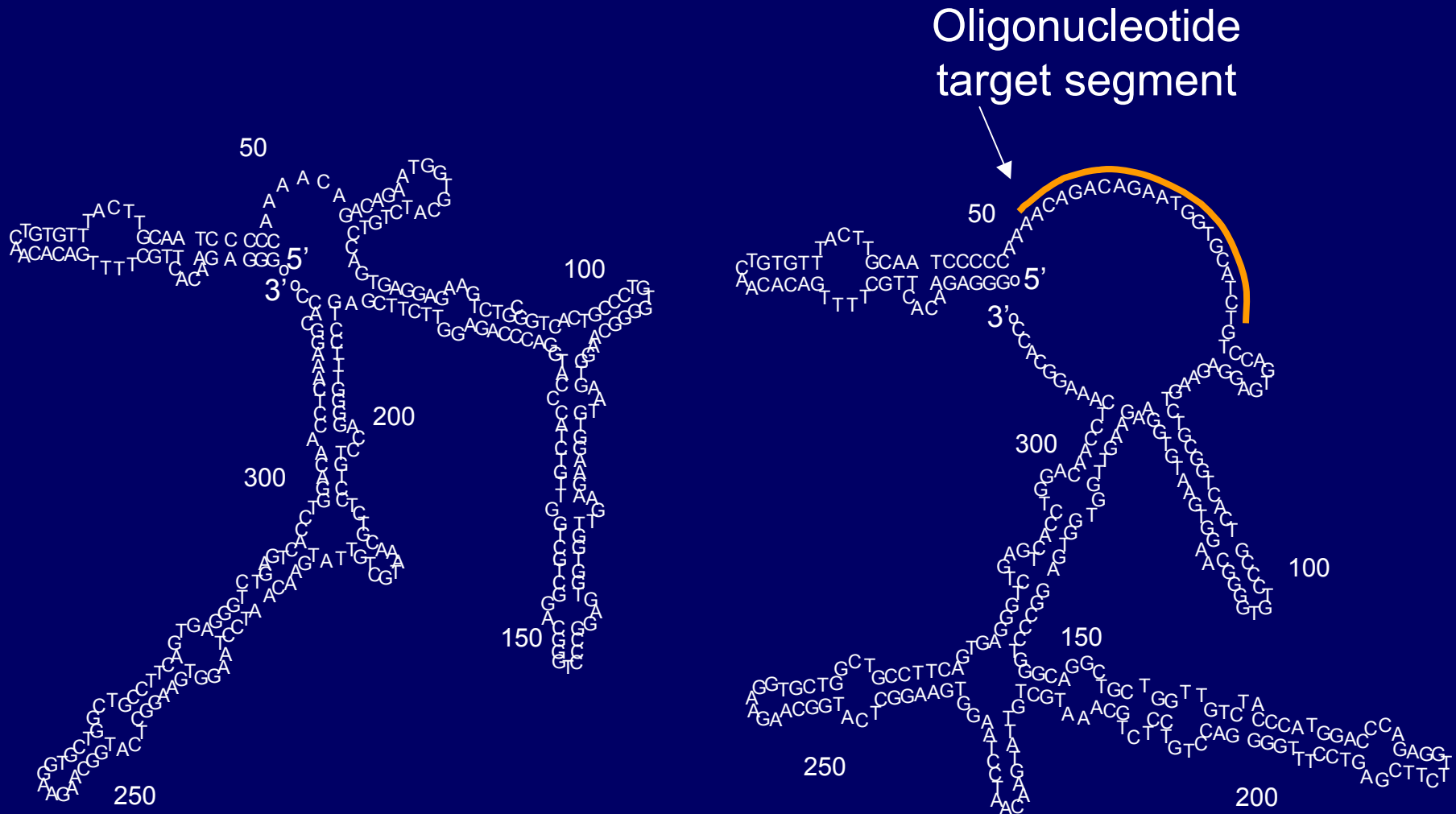


Primary Sequence Dictates mRNA 2° Structure



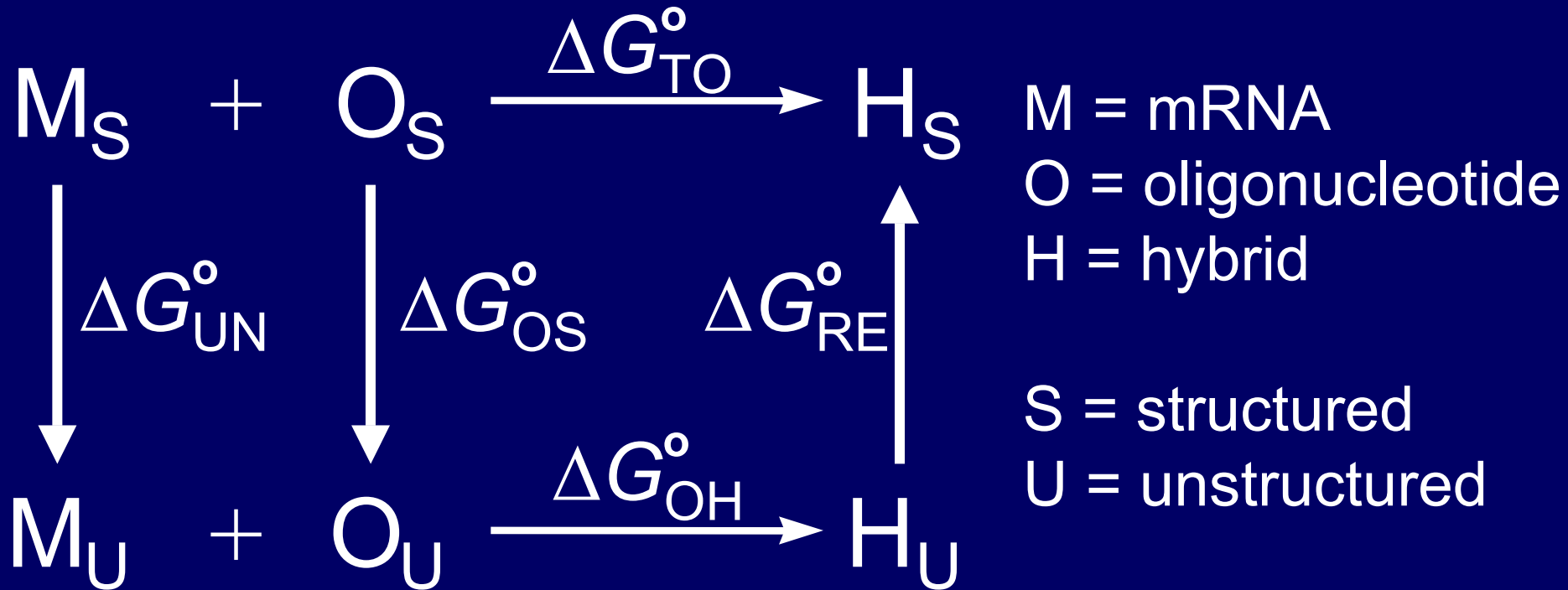
Structures predicted by free-energy minimization. Energies based on empirical data from short oligonucleotide pairs extrapolated to full-length RNAs.

Rearrangement due to Oligonucleotide



Possible Oligonucleotide Structure: T C G G A C A G
 C T G G G
 T G G A G

Thermodynamics of Hybridization



$$\Delta G_{TO}^{\circ} = \underbrace{\Delta G_{UN}^{\circ} + \Delta G_{RE}^{\circ}}_{\text{RNA Restruct.}} + \Delta G_{OS}^{\circ} + \Delta G_{OH}^{\circ}$$

Overall

**RNA
Restruct.**

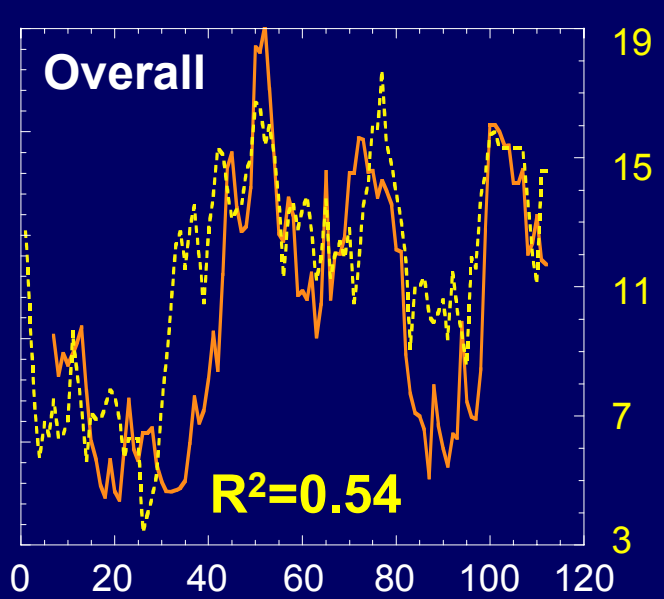
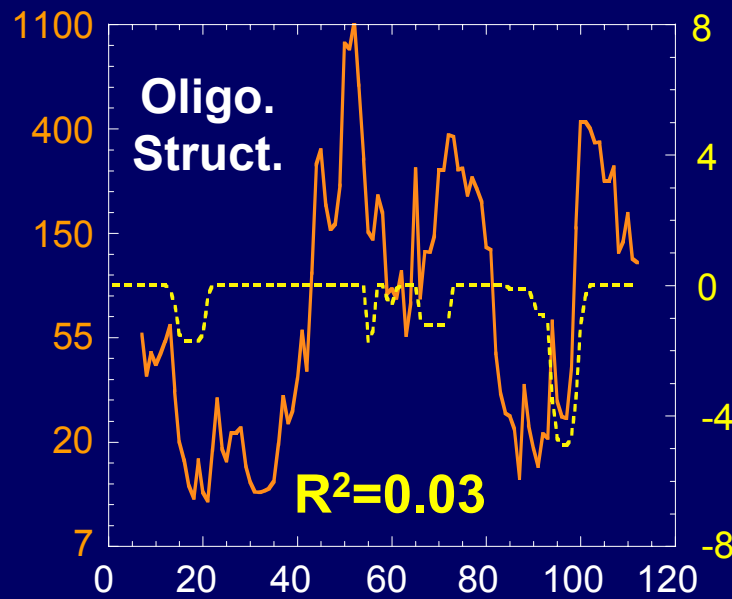
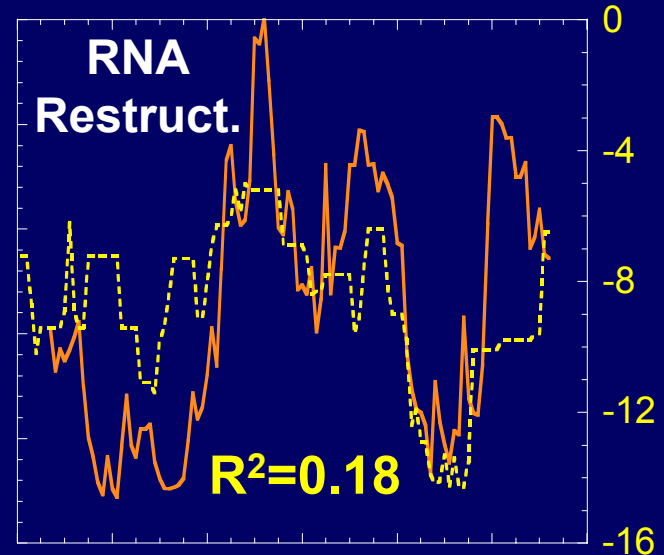
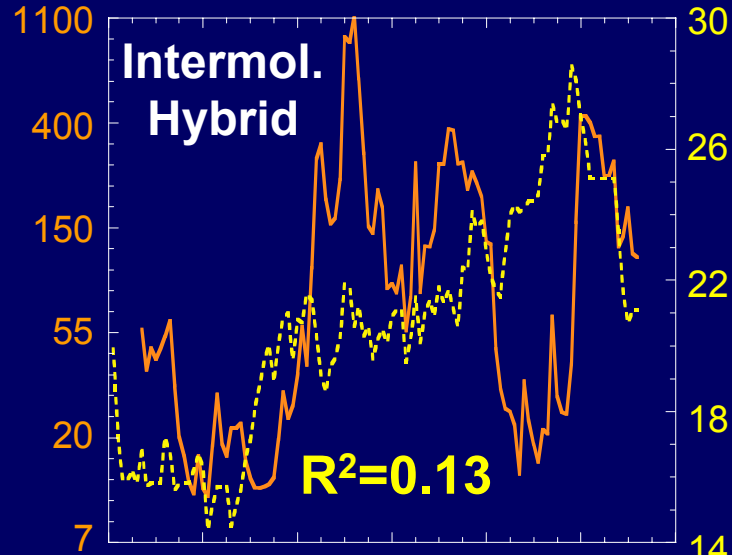
**Oligo.
Struct.**

**Intermol.
Hybrid**

Predictive Accuracy Requires All Components

Binding Affinity [arbitrary]

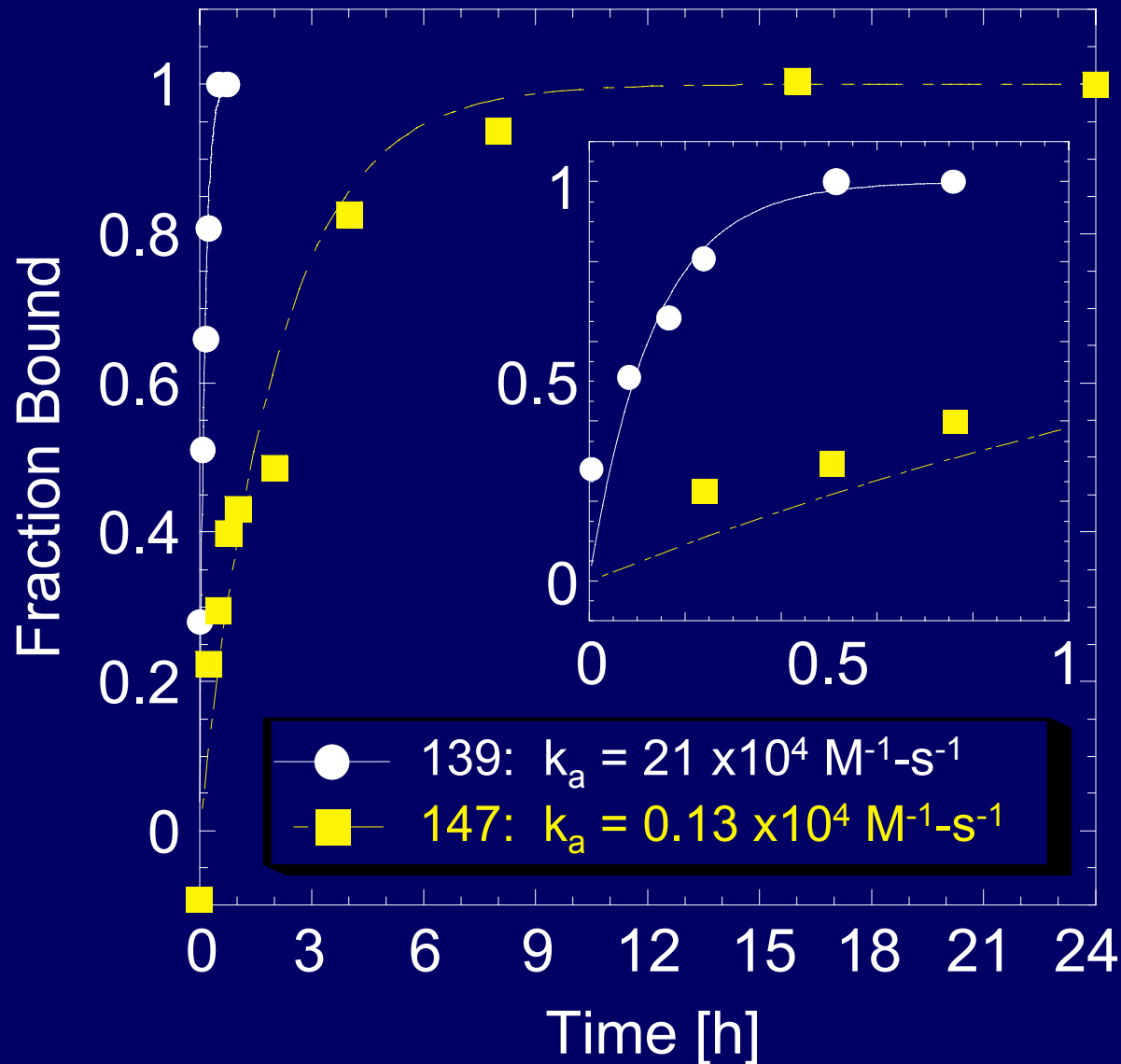
- [Free Energy Term] [kcal/mole]



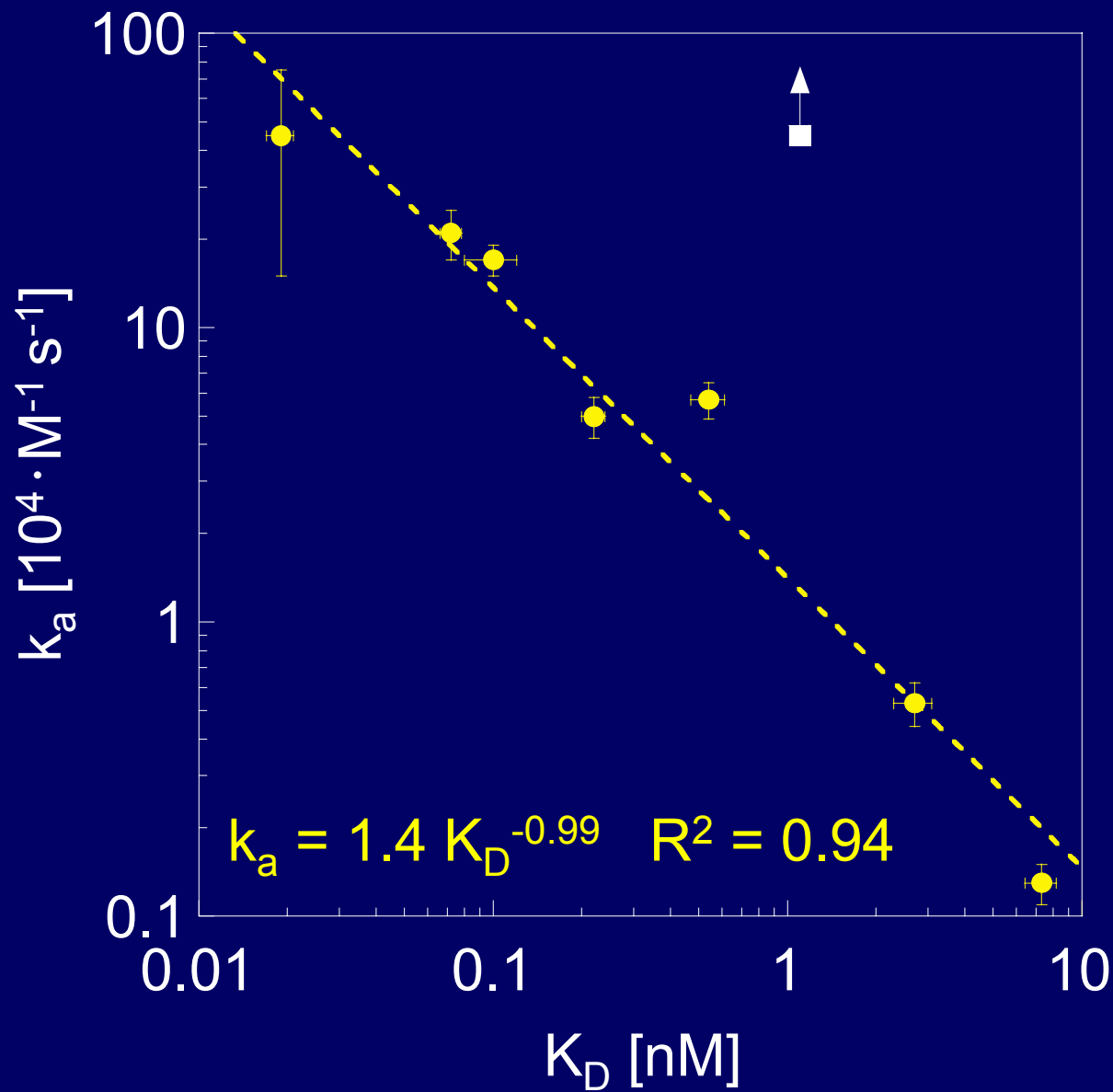
rabbit β -globin mRNA

Position

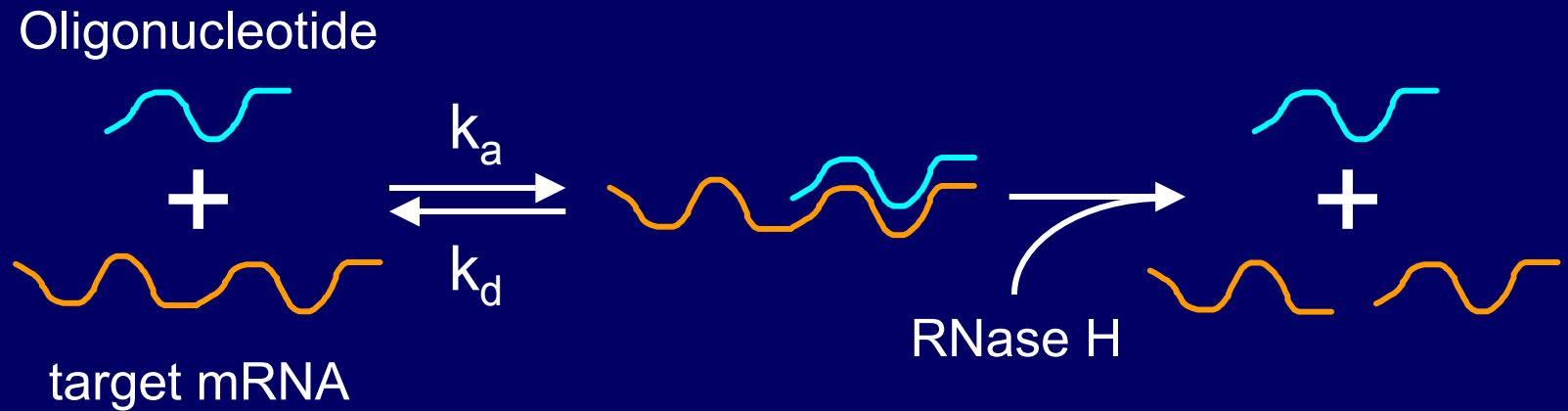
Association kinetics depend on target sequence



Affinity Determined Mainly by Association

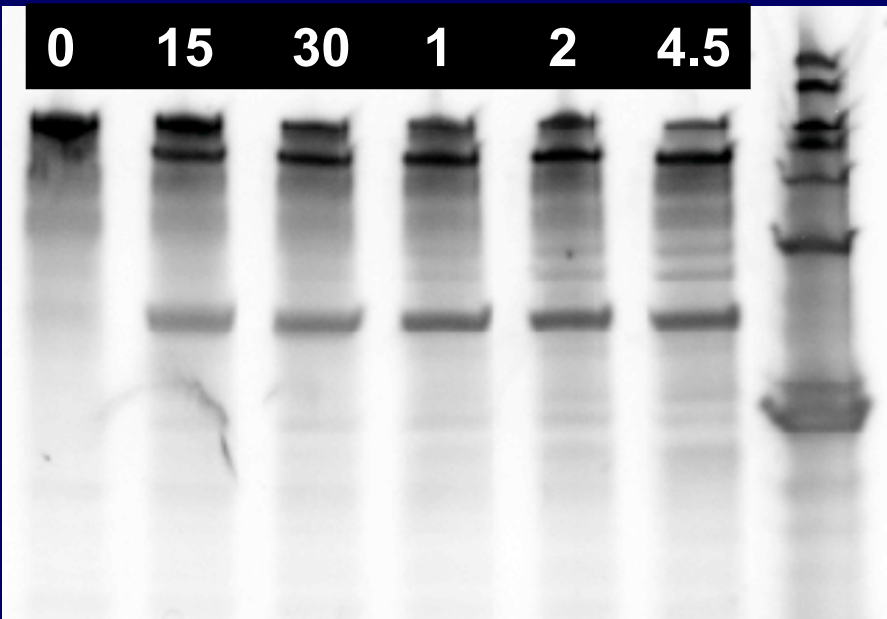


RNase H Cleavage



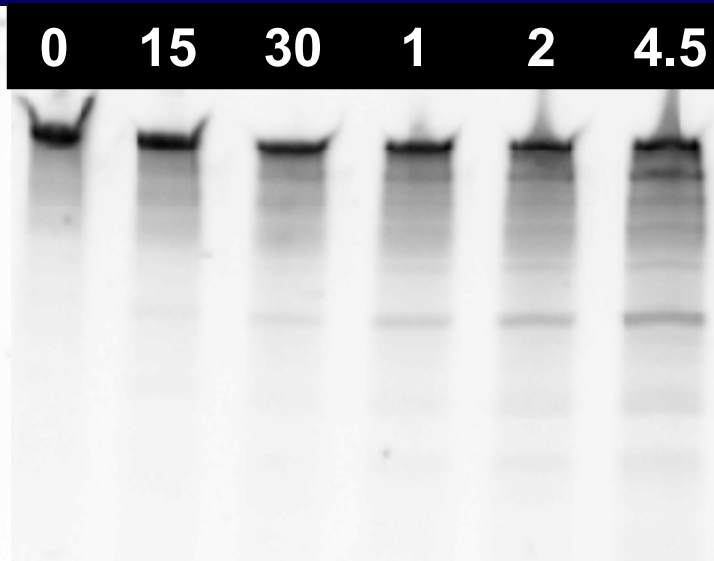
Time [h]

0 15 30 1 2 4.5



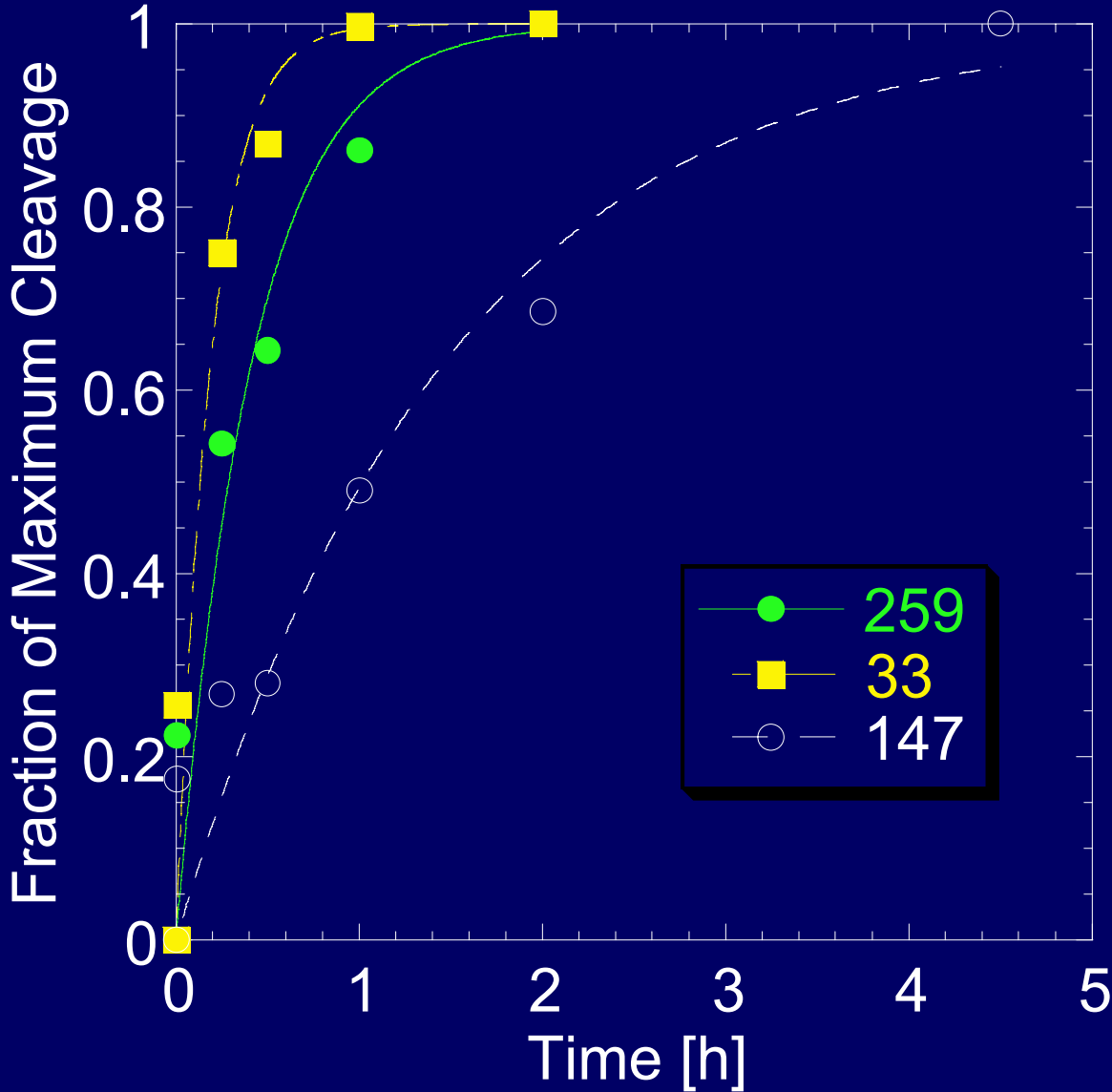
Time [h]

0 15 30 1 2 4.5



510
371/363
139/147

RNase H Cleavage Limited by Association Rate



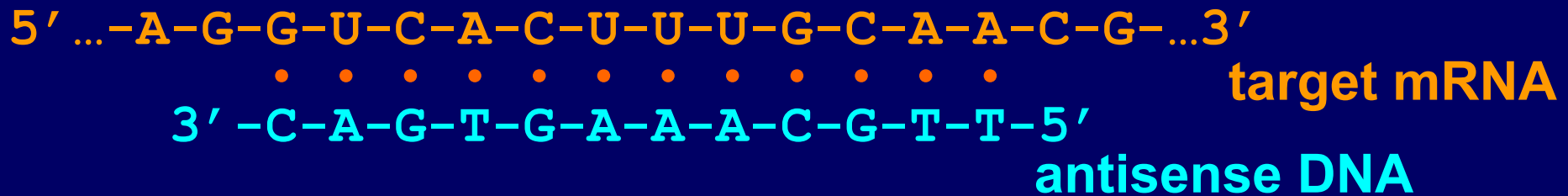
33: $k_a > 45 \times 10^4 M^{-1} s^{-1}$
 $k_{eff} = 6.5 \times 10^{-4} s^{-1}$

259: $k_a = 45 \times 10^4 M^{-1} s^{-1}$
 $k_{eff} = 4.6 \times 10^{-4} s^{-1}$

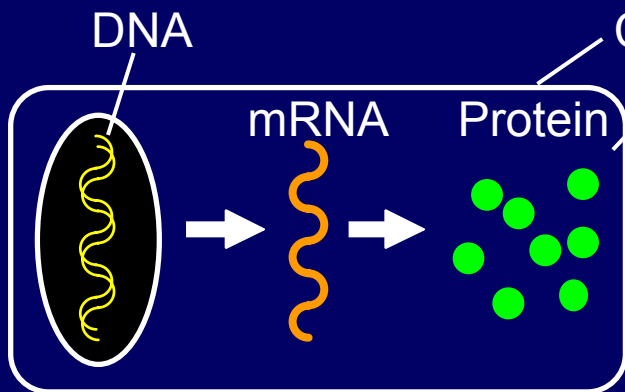
147: $k_a = 0.13 \times 10^4 M^{-1} s^{-1}$
 $k_{eff} = 0.47 \times 10^{-4} s^{-1}$

Principle of Antisense Activity

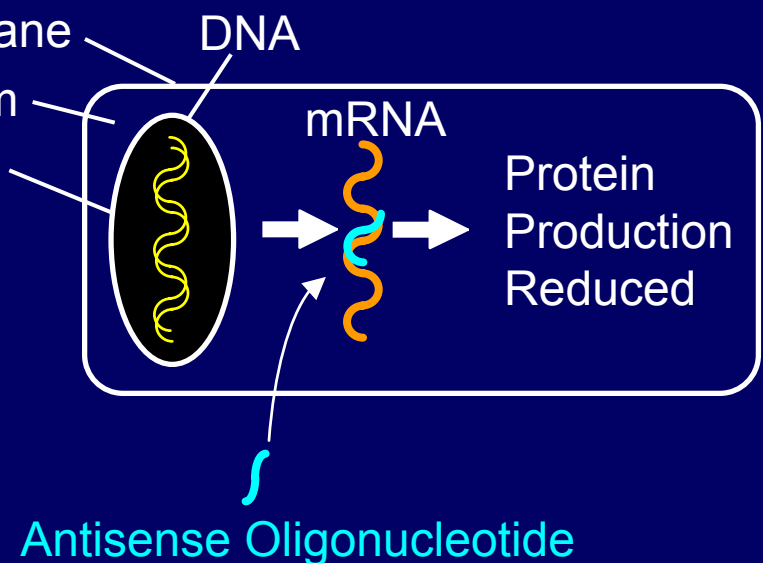
Inhibition by complementary base pairing



Normal Protein Production



Antisense Inhibition



Small RNAs

Breakthrough Online

For an expanded version of this section, with references and links, see www.sciencemag.org/content/vol298/issue5602/#special

Breakthrough

#1

The Winner

Just when scientists thought they had deciphered the roles played by the cell's leading actors, a familiar performer has turned up in a stunning variety of guises. RNA, long upstaged by its more glamorous sibling, DNA, is turning out to have star qualities of its own.

Small RNAs Make Big Splash

20 DECEMBER 2002 VOL 298 SCIENCE www.sciencemag.org

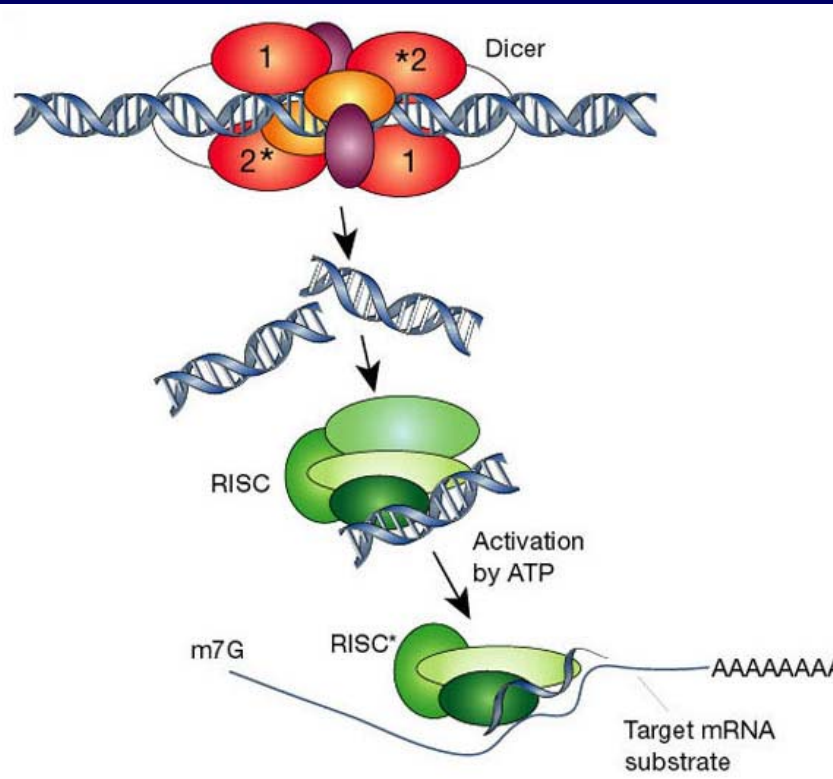
Small RNAs

Table 1. Processes affected by ncRNAs.

Process	Example	Function
Transcription	184-nt <i>E. coli</i> 6S 331-nt human 7SK 875-nt human SRA	Modulates promoter use Inhibits transcription elongation factor P-TEFb Steroid receptor coactivator
Gene silencing	16,500-nt human <i>Xist</i> ~100,000-nt human <i>Air</i>	Required for X-chromosome inactivation Required for autosomal gene imprinting
Replication	451-nt human telomerase RNA	Core of telomerase and telomere template
RNA processing	377-nt <i>E. coli</i> RNase P 186-nt human U2 snRNA	Catalytic core of RNase P Core of spliceosome
RNA modification	102-nt <i>S. cerevisiae</i> U18 C/D snoRNA 189-nt <i>S. cerevisiae</i> snR8 H/ACA snoRNA 68-nt <i>T. brucei</i> gCYb gRNA	Directs 2'-O-ribose methylation of target rRNA Directs pseudouridylation of target rRNA Directs the insertion and excision of uridines
RNA stability	80-nt <i>E. coli</i> RyhB sRNA Eukaryotic miRNA?	Targets mRNAs for degradation? Targets mRNAs for degradation?
mRNA translation	109-nt <i>E. coli</i> OxyS 87-nt <i>E. coli</i> DsrA sRNA 22-nt <i>C. elegans</i> <i>lin-4</i> miRNA	Represses translation by occluding ribosome binding Activates translation by preventing formation of an inhibitory mRNA structure Represses translation by pairing with 3' end of target mRNA
Protein stability	363-nt <i>E. coli</i> tmRNA	Directs addition of tag to peptides on stalled ribosomes
Protein translocation	114-nt <i>E. coli</i> 4.5S RNA	Integral component of signal recognition particle central to protein translocation across membranes

RNA Interference

1.)



2.)

3.)

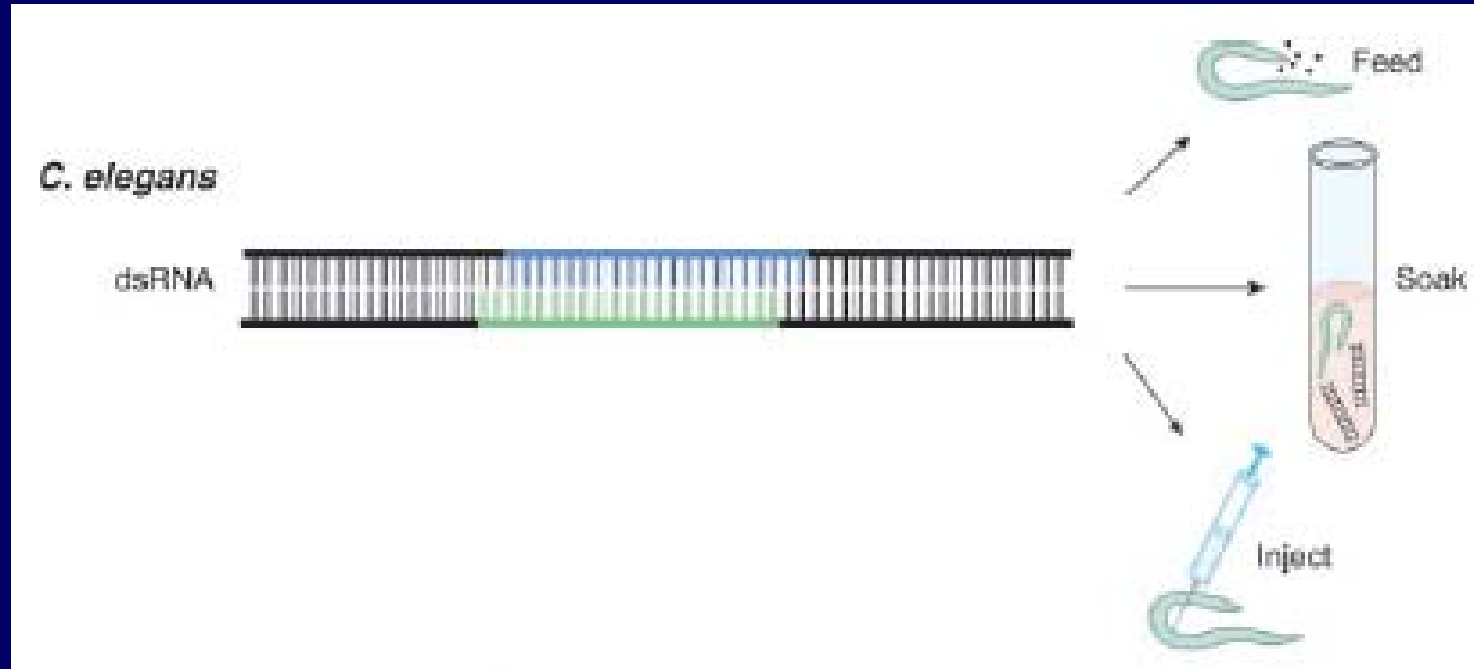
1.) Upon recognition of dsRNA, Dicer dimerizes to cleave the dsRNA, generating siRNAs.

2.) Free siRNAs initiate RISC formation (shown in green).

3.) RISC relies on homology to direct specific cleavage of the substrate.

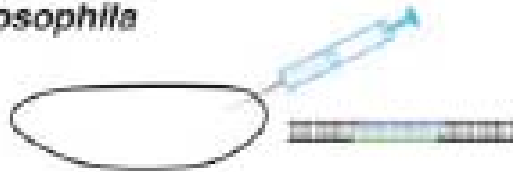
Subsequent events in the pathway have not yet been characterized

RNA Interference



RNA Interference

Drosophila



Inject embryos
with dsRNA

Or



Create transgenic flies that
express dsRNA hairpin

Cultured *Drosophila* cells

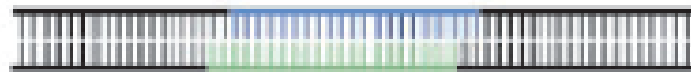


Soak directly in medium
containing dsRNA

RNA Interference

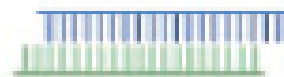
Cultured Mammalian cells

Embryonic cultured cells or oocytes or early embryos



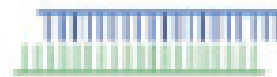
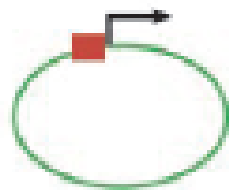
Transfect or inject dsRNA

Differentiated cultured cells or whole organisms (in vivo)



Transfect synthetic siRNA

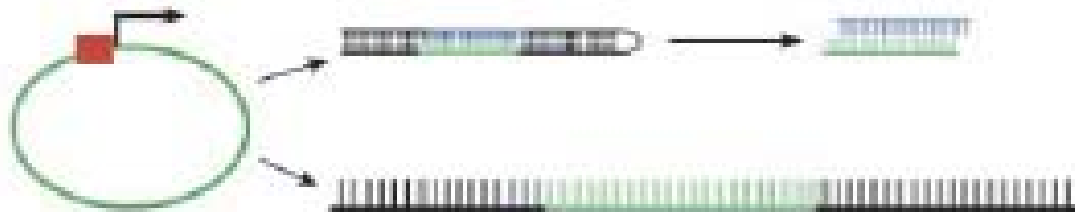
Or



Transfect plasmids expressing miRNA precursor-like stem-loop to generate siRNA in cells

RNA Interference

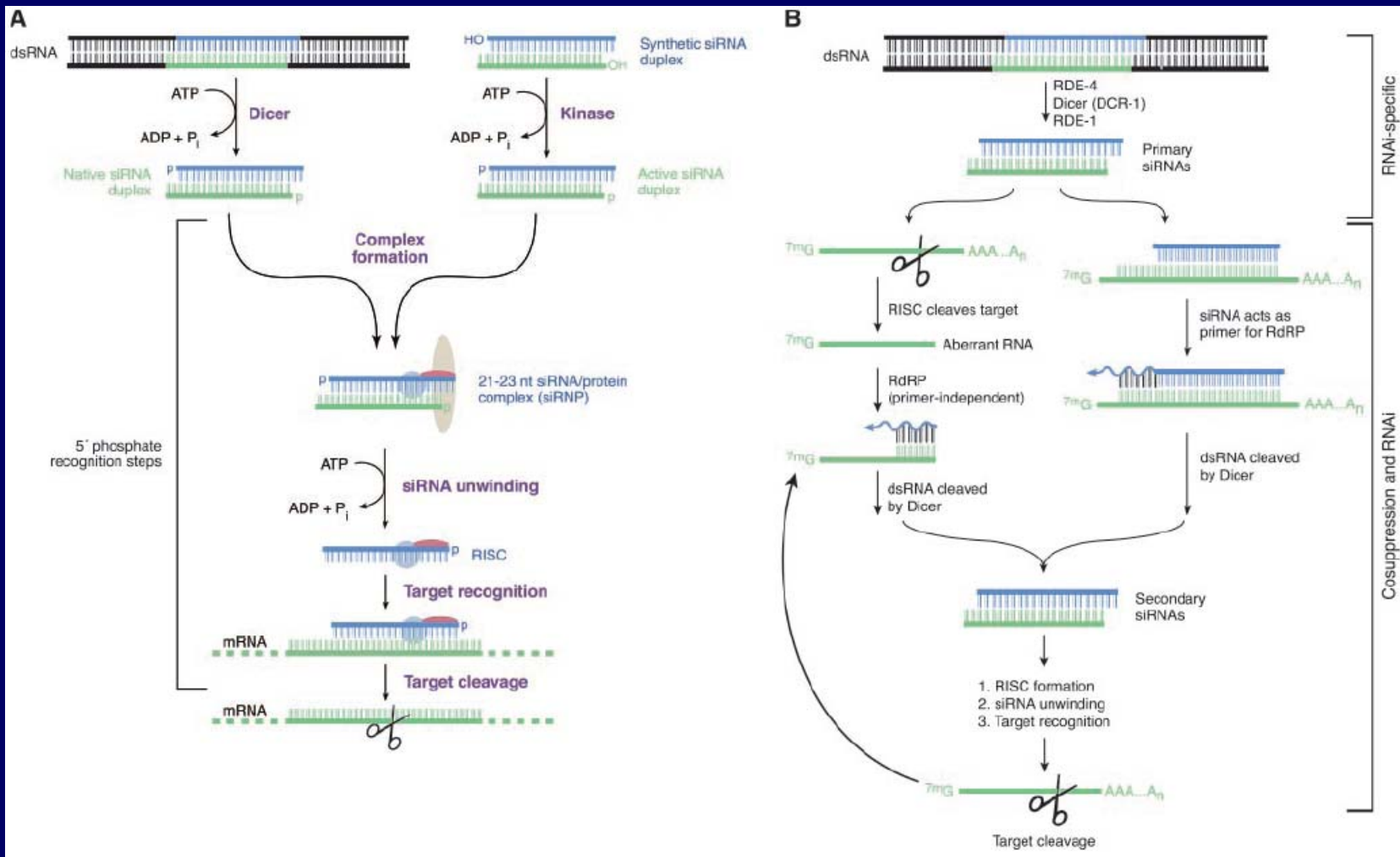
Plants



Overexpress dsRNA or a dsRNA hairpin from a transgene

Overexpress single-stranded RNA from a transgene or virus to trigger co-suppression

RNA Interference



RNAi in the Clinic?

Success of RNAi in the treatment of human disease will depend on the rigorous design of the siRNA strands used as well as the clear understanding of the mechanism by which these molecules exert their action.

RNAi in the Clinic?

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RNAi is today where antisense was 10 years ago.

