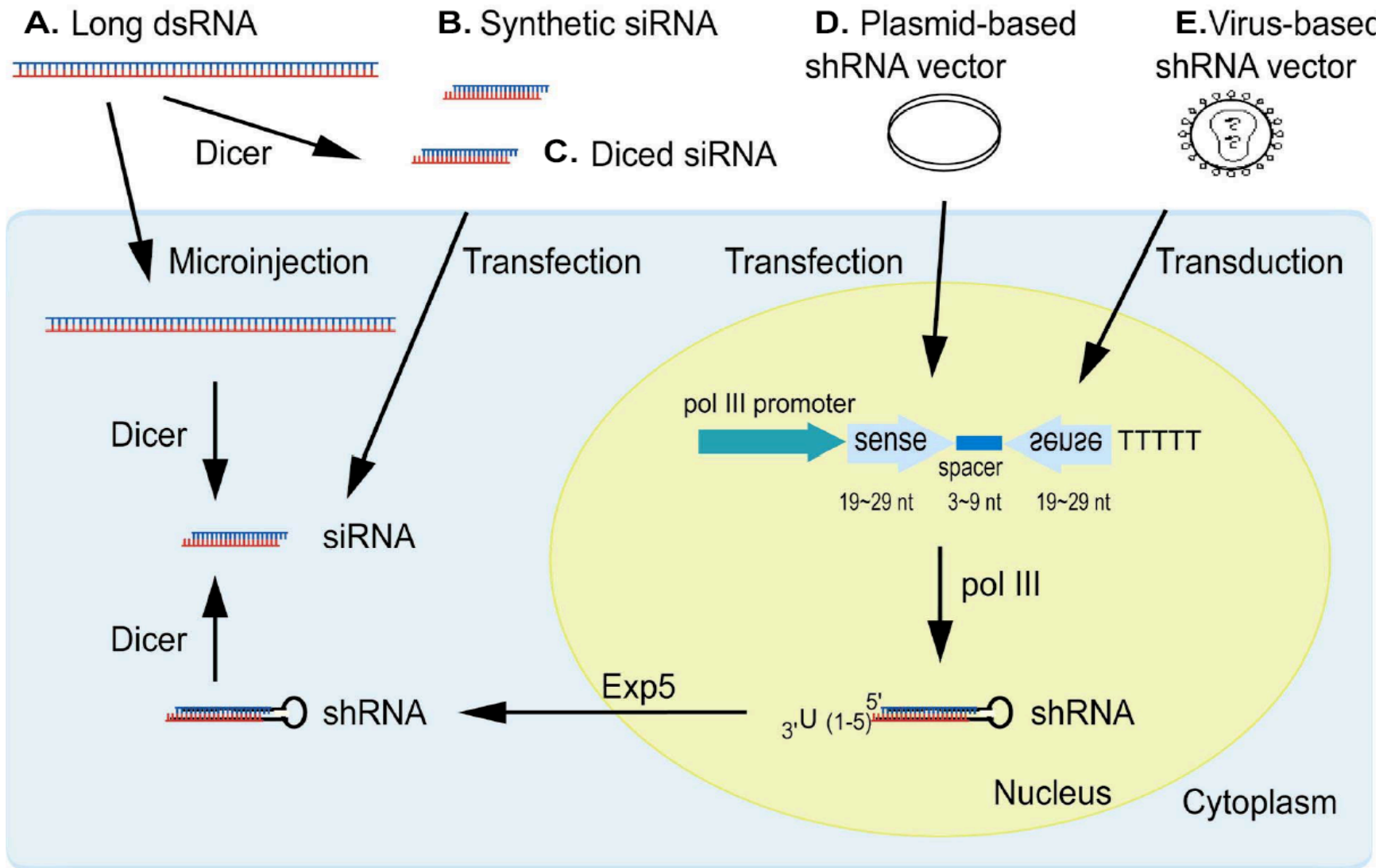# siRNA Delivery & Processing

siRNA Design

- Initial use of longer dsRNA lead to a non-specific Type I interferon response (widespread changes in protein expression→apoptosis)

- Dr. Thomas Tuschl's lab discovered that RNAi is mediated by 21 and 22 nt RNAs

- Also discovered the important characteristics needed by the RNAs

siRNA Expression

- For transient expression: duplex RNA can be delivered to the cell

- For a stable expression: a vector containing the DNA to produce a hairpin RNA

- The vector may be plasmid, retrovirus, adenovirus

siRNA Delivery

- For cell culture
  - Lipid-based transfection
  - Electroporation
- *In vivo*
  - Lipid-based
  - Conjugations
    - Bacterial phage RNA
    - Cholesterol
    - Atelocollagen
  - Viral systems (ie retrovirus & adenovirus)

Applications for siRNA

- Basic research
  - Determining protein function
  - Easier than a knockout and may be used for partial knockdowns
- Clinical research
  - Cancer, hypercholesterolemia, infections, developmental defects

Uses of siRNA

- Gene knockdowns
  - Look at function/phenotype of a gene
- Therapeutic techniques
  - Anti viral
  - Anti cancer
  - Anti neurological diseases
  - Others

microRNA therapeutics					
SPC3649	Santaris Pharma	Phase I	miR122	Hypercholesterolemia and hepatitis C	Intravenous
RNA therapeutics					
Bevasirinab sodium (Cand5)	Opko	Phase III Phase II	VEGF	Age-related macular degeneration	Naked siRNA, intravitreal
AGN-211745	Allergan—Sirna Therapeutics/ Merck	Phase II	VEGFR1	Age-related macular degeneration	Naked siRNA, intravitreal
ALN-RSV01	Alnylam Pharmaceuticals/Kyowa Hakko Kogyo (Asia)	Phase II	Respiratory syncytial virus	Respiratory syncytial virus infection	Naked siRNA, intranasal and inhalation

Sepp-Lorenzino & Ruddy. Clin Pharmacol Ther. 2008 Sep 17

siRNA – designing the assay

- dsRNAs need to be <30 bp in length
  - Why?
- Well-designed siRNAs can result in >90% reduction in target RNA
- 21nt dsRNAs most effective
- Sequence-specificity important
  - Single bp-mismatches reduce silencing capability
- Many will make 3-4 siRNAs – test all and go w/ best
- Deliver by injection or transfection
- Vectors becoming more popular