BICH/GENE 431 KNOWLEDGE OBJECTIVES

Chapter 18 – Regulatory RNAs
Bacterial small RNAs (sRNA)
- 80-110 nt
- Hfq protein facilitates binding of sRNA to target RNA
- Cause degradation of target or control translation
- Unmask RBS to activate translation (DsrA/RprA with rpoS in E. coli)
- Or, bind to RBS to inhibit translation (OxyS with rpoS in E. coli)
Riboswitch RNAs in bacteria, fungi, plants
- general structure with aptamer region and expression platform; basic mechanism
- attenuation with SAM on riboswitch RNA in B. subtilis
- blocking of RBS upon binding SAM riboswitch in B. subtilis
RNA interference (RNAi) in eukaryotes (not S. cerevisiae)
- siRNA or miRNAs 21-23 nt long after processing
- differences between siRNAs and miRNAs
- function of Dicer ribonuclease
- function of RISC complex
- Slicer activity by Argonaute protein in RISC
- Either target mRNA degradation or inhibition of translation
- Can target genes in chromatin for transcriptional silencing too
- RdRP (RNA-dependent RNA polymerase): role in RNAi
Biosynthesis of microRNAs
- encoded in genes and transcribed by RNAP II (exons, introns, UTR)
- approx. 120 in C. elegans, 250-300 in humans; highly conserved
- pri-miRNA (primary microRNA) cut by Drosha nuclease in nuclease
  part of microprocessor complex
  Drosha recognizes structure, not sequence of pri-miRNA; cuts both sides of
  lower stem to give pre-miRNA; exported to cytoplasm
- Dicer ribonuclease processes to give ds miRNA that is loaded into RISC
- Some pre-miRNAs contain two different miRNAs on opposite sides of stem
RNA-induced transcriptional silencing (RITS)
- understand example of centromeric silencing found in fission yeast (S. pombe)
- also important in other organisms, esp. plants
Use of RNAi to manipulate gene expression
- knock-down of gene expression, not genomic knock-out, such as in mice, yeast
- understand example of how it's done in C. elegans
- shRNAs (short hairpin RNAs) used in human cells: cut by Dicer in cells and
  loaded into RISC; introduced into cells as genes in lentivirus vectors and
  transcribed into shRNAs
RNAi and human disease
- many miRNAs important in cancer; control genes for cell cycle progression,
  apoptosis

- miRNAs can control expression of potentially oncogenic genes or tumor suppressor genes; too little or too much miRNA can cause misexpression of these genes and lead to cancer
- Fragile X mental retardation: FMR1 protein (mutated in Fragile X) is component of RISC complex

X chromosome inactivation in mammals controlled by regulatory RNAs (not RNAi)
- Why important to inactivate X? Dosage compensation
- Females are mosaics – why? Good example is calico cat.
- Xist RNA coats X chromosome; recruits HDACs, DNA methylases
- Tsix RNA is negative regulator to help determine which X is inactivated