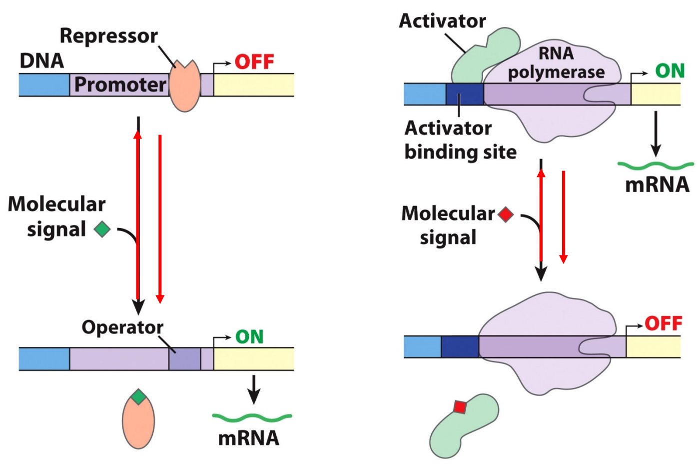
Chapter 28: Gene regulation

Principles of gene regulation
- Multiple potential points of regulation
- Energetic and efficiency concerns
- Transcriptional vs. translational control

Gene regulation mediated by proteins
- Transcription: SOS response
- Translation: ribosomal protein synthesis

Gene regulation mediated by RNA
- Regulatory RNAs in bacteria: sRNA, Riboswitch
- Regulatory RNAs in eukarya: miRNA/siRNA

Control at the level of transcription



Induction of the SOS response in *E.coli*

control of distantly spaced genes- not operon-based

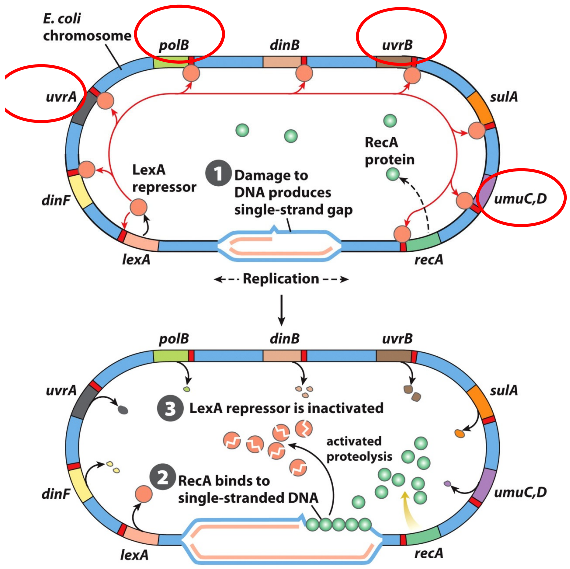


Figure 28-20
Lehninger Principles of Biochemistry, Sixth Edition

LexA and RecA:
central role
- autoproteolysis
- only ssDNA-bound RecA

links damage to SOS

Control at the level of translation

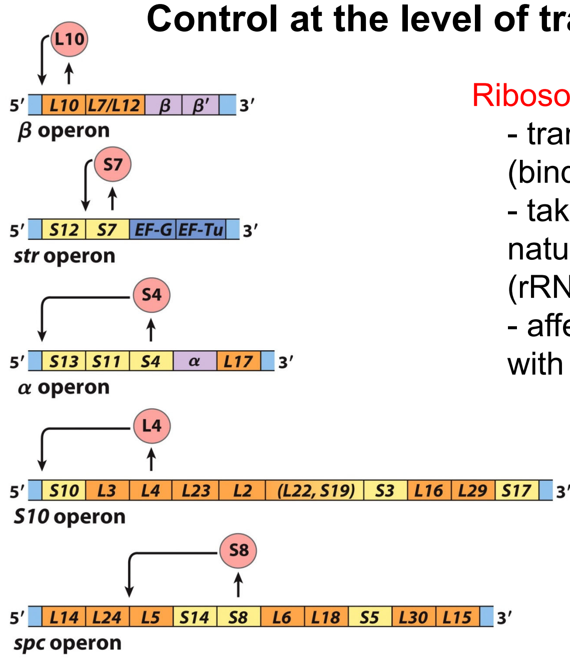


Figure 28-21
Lehninger Principles of Biochemistry, Sixth Edition

Ribosomal protein operons:
- translational repressors (bind mRNA)
- take advantage of natural affinity for RNA (rRNA and mRNA)
- affect >1 gene/operon with single repressor

Requires controlled balance: rRNA vs r-proteins

r-protein must be >rRNA for translational repression- how does this happen?

nutrients are low, ↓aa synthesis, ↑ uncharged-tRNA

activates the **Stringent response**- effectively halts rRNA synthesis

forms **ppGpp**- 2nd messenger

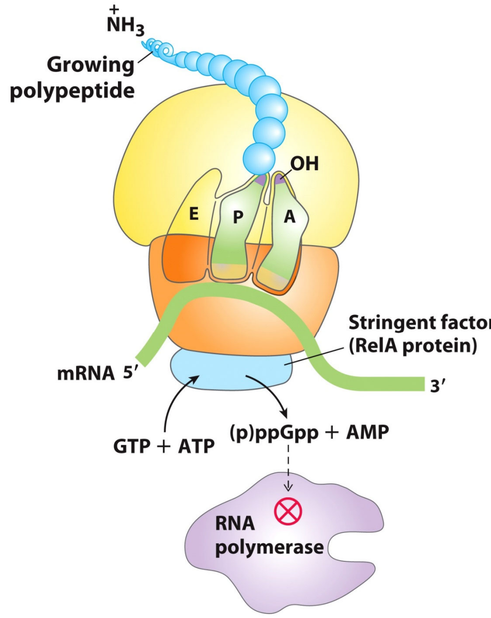


Figure 28-22
Lehninger Principles of Biochemistry, Sixth Edition

Gene regulation mediated by RNA

Regulatory RNAs in Bacteria

- sRNAs acting by base pairing
- Riboswitches: sensing metabolites or nutrients

Regulatory RNAs in Eukarya

- miRNA/siRNA: the RNA interference pathway
- an abundance of ncRNAs- transcripts of unknown function??

Small RNA regulators (sRNAs) in bacteria

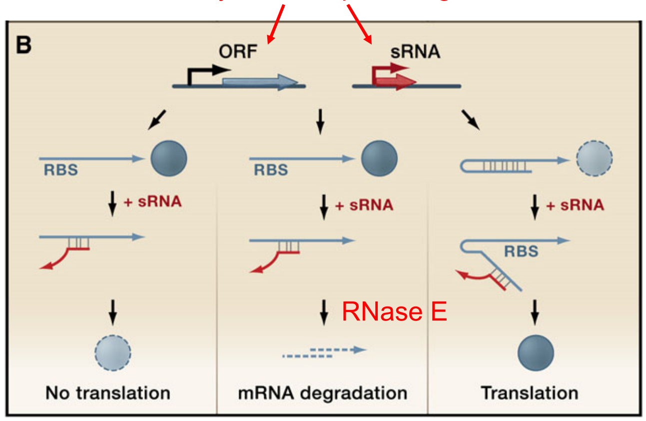
Well-known long before miRNA/siRNA
- First discovered in early 1980's
- More now: more genomes, computational approaches, transcriptional profiling

Some affect activity of proteins

Selecting targets for regulation by base pairing
often negative regulation
translational inhibition, mRNA degradation or both
often requires RNA chaperone: Hfq

General model for sRNA regulation

encoded by different parts of genome



multiple targets are possible
due to limited complementarity
(20-25 nt)

Waters and Storz, Cell 136, 615–628

sRNA regulation of the *rpoS* gene

general stress response-
Low nutrients, stationary phase

oxidative stress response-
don't turn on general stress genes

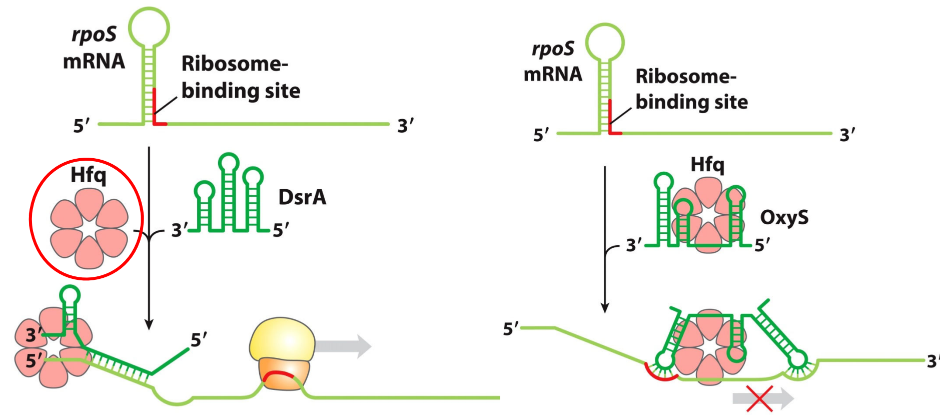


Figure 28-23a
Lehninger Principles of Biochemistry, Sixth Edition
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Figure 28-23b
Lehninger Principles of Biochemistry, Sixth Edition
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make rpoS (σ^S)

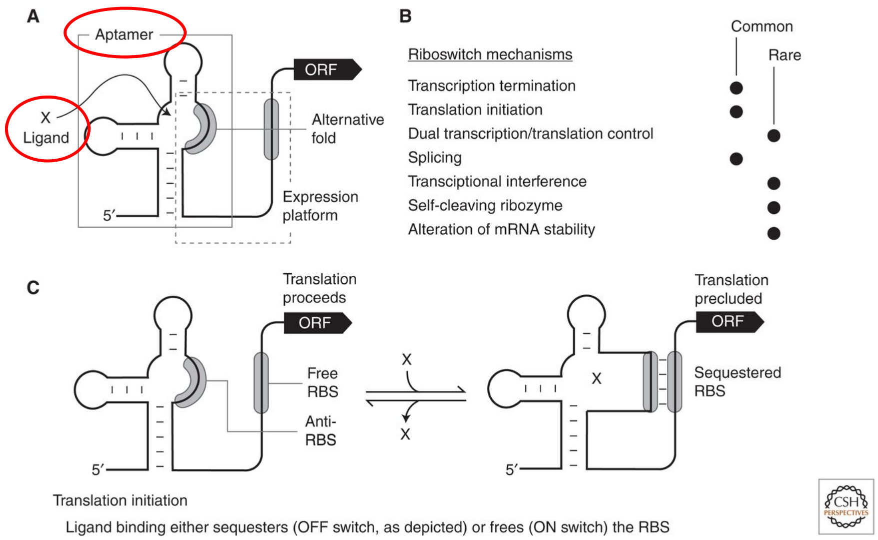
Riboswitches: response to effector molecules

Abundant mechanism in Bacteria
- up to 4% of *B. subtilis* genes

<u>Two domains</u>
- Aptamer: binds metabolite
- Expression platform: causes change in expression

Sensitive to changes in concentration of metabolites
- effectors: SAM, lysine, glycine, purines, B12, GlcN6P…

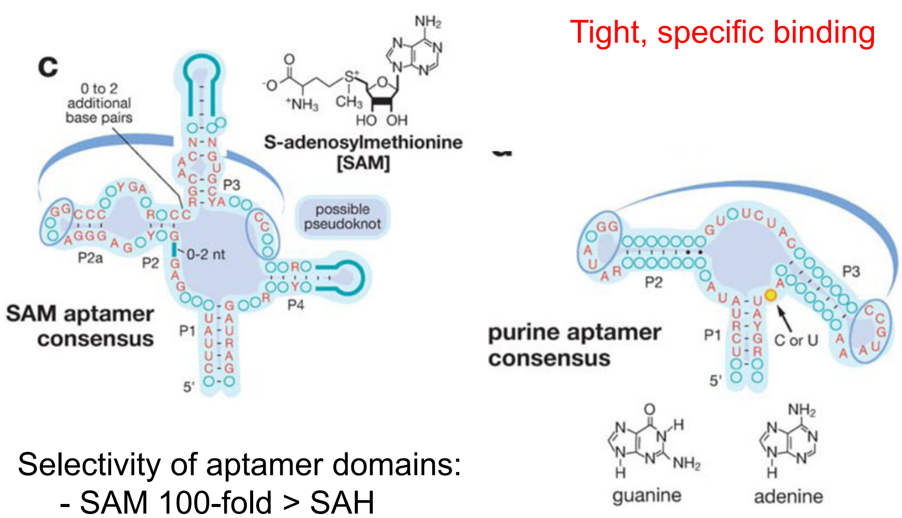
General principles of riboswitch gene regulation



Breaker, *Cold Spring Harb Perspect Biol* 2018

Aptamer domains: highly conserved sequences

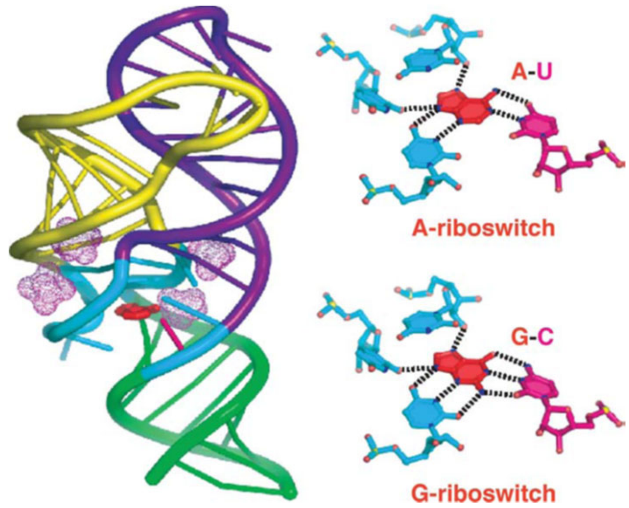
Tight, specific binding



Selectivity of aptamer domains:
- SAM 100-fold > SAH
- 10,000-fold < SAC

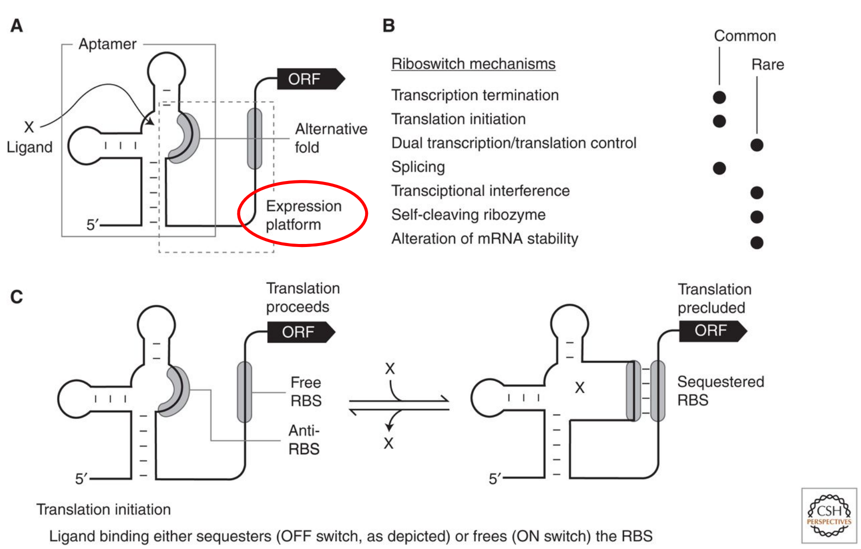
Winkler and Breaker, *Ann Rev Micro* 2005

Purine riboswitch: recognition by base pairing



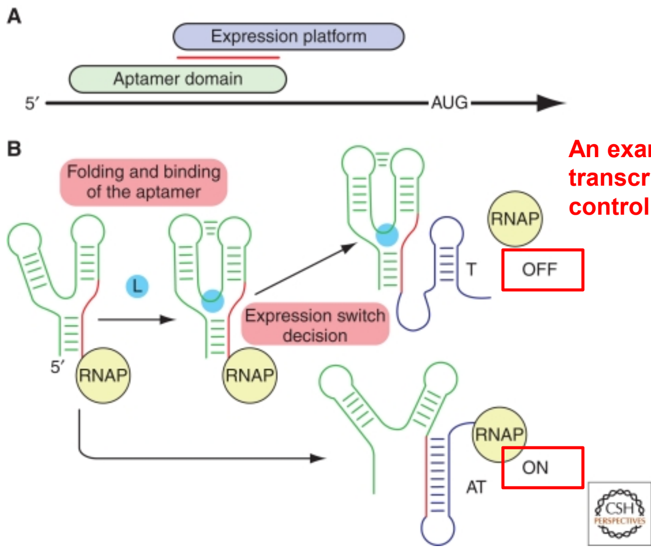
Winkler and Breaker, *Ann Rev Micro* 2005

General principles of riboswitch gene regulation



Breaker, *Cold Spring Harb Perspect Biol* 2018

Expression platform: rearranging RNA structures



An example of transcriptional control

Garst et al, *Cold Spring Harb Perspect Biol* 2011

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Regulatory RNAs in Eukarya

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Eukaryal small RNAs (miRNA, siRNA)

Lessons from petunias…

"Co-suppression"

Now known as RNA interference (RNAi)

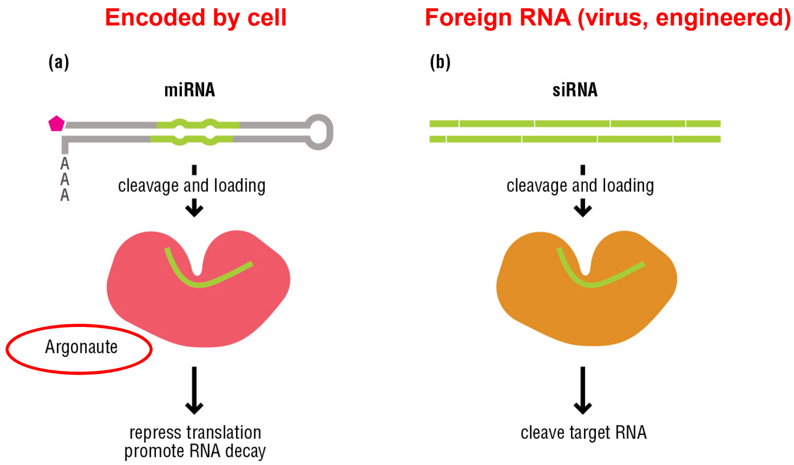
The RNA interference (RNAi) pathway

First described in *C. elegans*: Fire and Mello, 1998
- widespread in higher eukaryotes
- short RNAs (<26nt) escape degradation

Many functions in vivo
- developmental timing
- protection from viral attack (particularly plants)
- control activity of transposons
- formation of heterochromatin

miRNA vs siRNA:
- miRNA: control of endogenous genes (*C. elegans*)
- siRNA: defenders from foreign nucleic acid invasion
- common mechanism/themes for both

Gene silencing by RNA interference

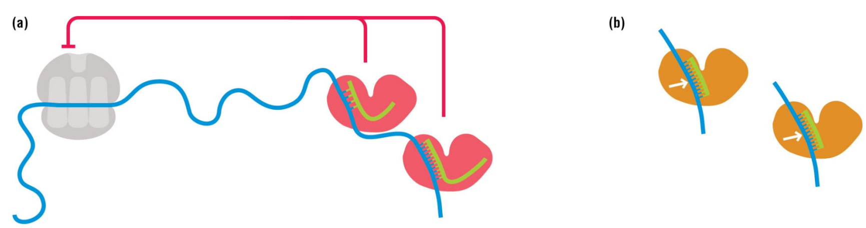


RISC complex (RNP): RNA-induced silencing complex

From Molecular Biology: Craig et al, Oxford University Press, 2nd Ed

Gene silencing by RNA interference

RISC complex: affecting translation or stability



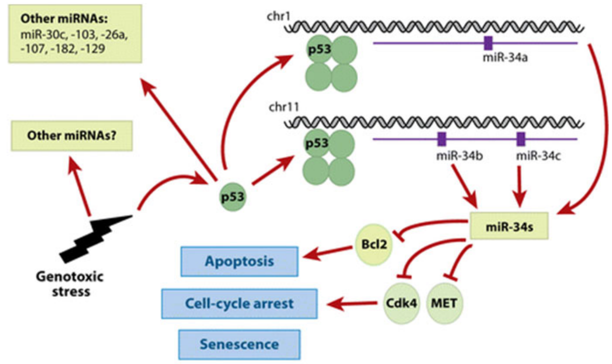
Imperfect pairing with target:
- i.e. miRNA/human mRNA
- leads to translation repression

Perfect pairing with target:
- i.e. siRNA/viral RNA
- leads to cleavage

From Molecular Biology: Craig et al, Oxford University Press, 2nd Ed

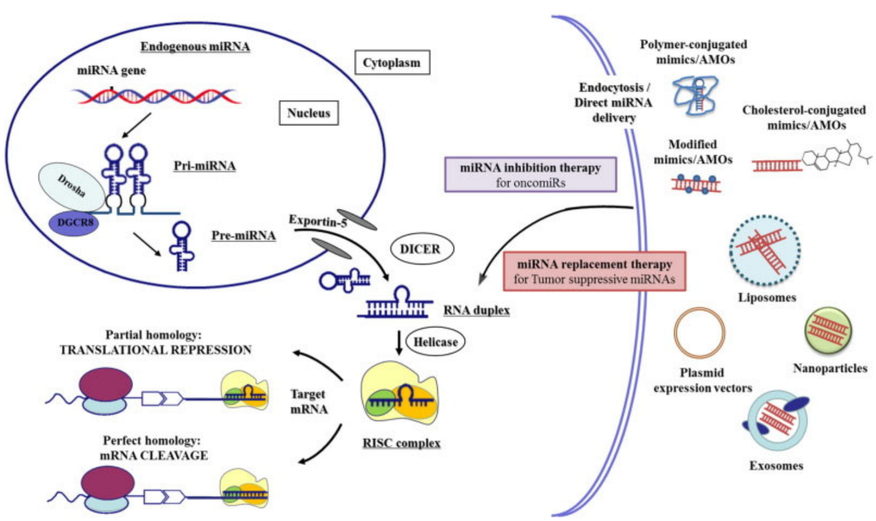
miRNA and disease: key regulatory players

Example: miR-34 family of tumor suppressors



Lee YS, Dutta A. 2009.
Annu. Rev. Pathol. Mech. Dis. 4:199–227

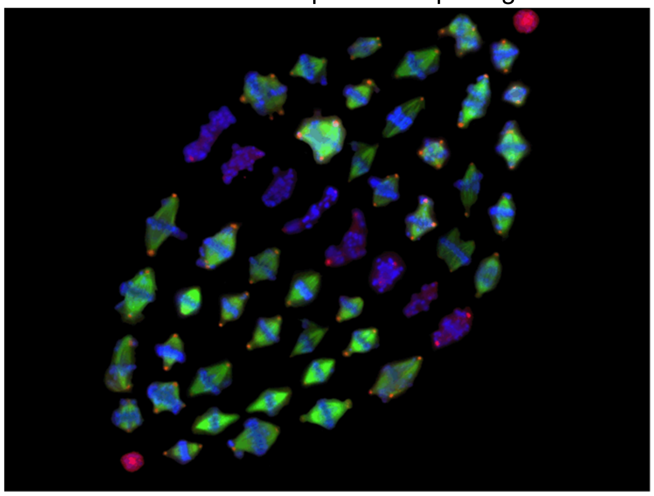
miRNA and disease: potential therapeutics



Shah et al, *EBioMedicine* 2016

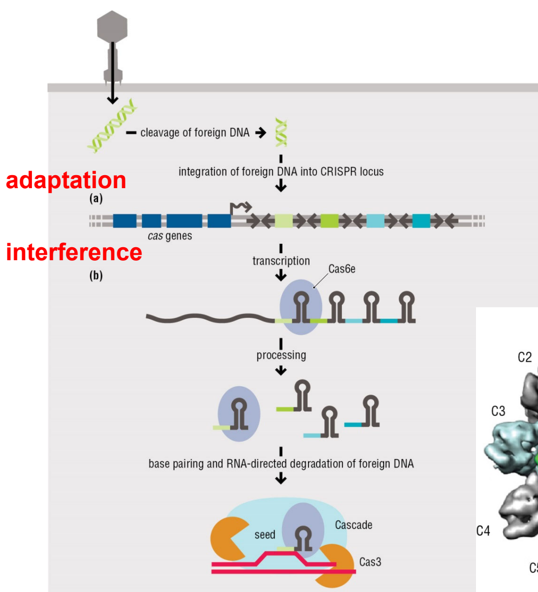
Or just use it for fun…

51-different RNAi induced mitotic spindle morphologies in Drosophila cells

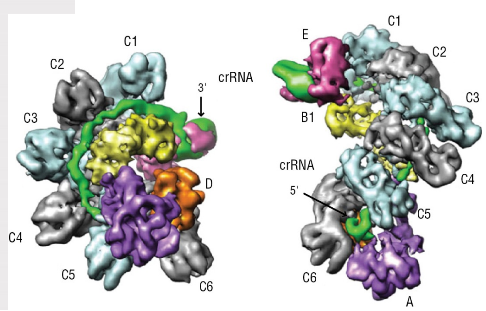


Goshima et al, Science 2007

E. coli CRISPR-Cas system

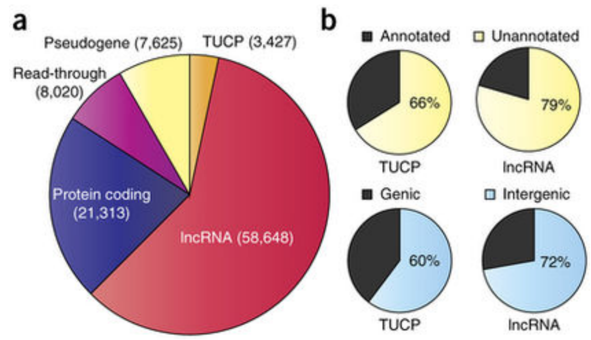


Cascade complex (RNP):
RNA: crRNA
Proteins: Cas proteins



The tip of the iceberg: ncRNAs in eukaryotes

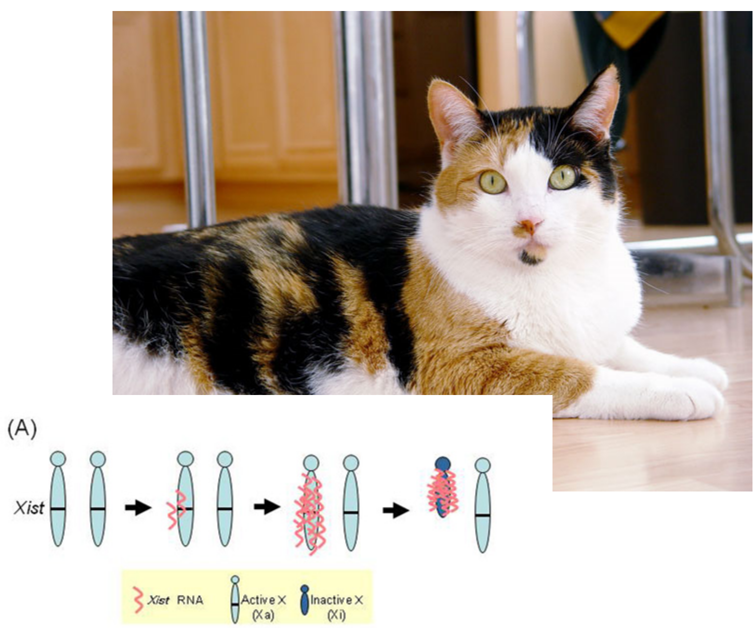
"The landscape of long noncoding RNAs in the human transcriptome"
(RNA from tumors, normal tissues and cell lines)



68% of human genes!

Iyer et al, N*ature Genet* 2015

Xist: inactivation of the X-chromosome in females



Hoki et al, Development 2009