Biology for nanoscientists

Frontiers of Nanotech

Beth Traxler Thur, 30/3/06

Goals for this lecture

50% of class has had 0 or 1 Bio class

- Overview of cell size, shape and organization
- Summarize some issues/questions in synthesis and assembly of cellular structures
- Provide forum for posing (and answering?) questions

Scale of biological systems

Compare:

C—C 0.15 nm

Glucose 0.9 nm

Ribosome 30 nm

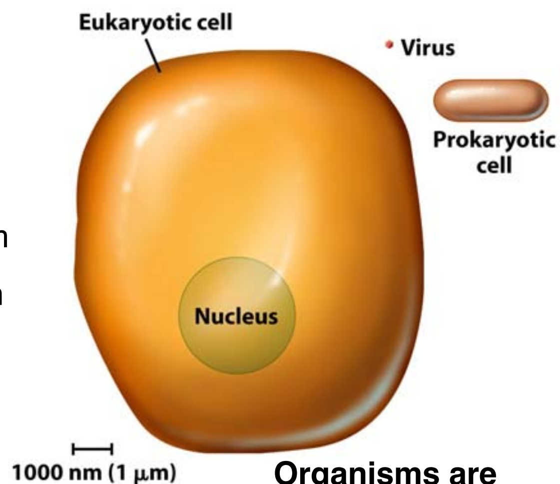


Figure 2-3c Brock Biology of Microorganisms 11/e

Organisms are not on nanometer scale!!

The challenge of biological systems

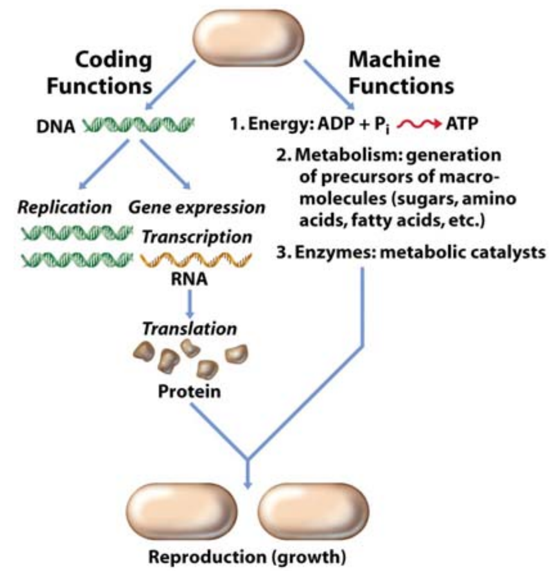
Despite their unyieldy size, thinking about whole organisms is fun and worthwhile!!

- Nanotechnologists can harness the power of biological systems to develop or assemble new technologies
- Subcellular structures and machines provide powerful examples of nanotechnology

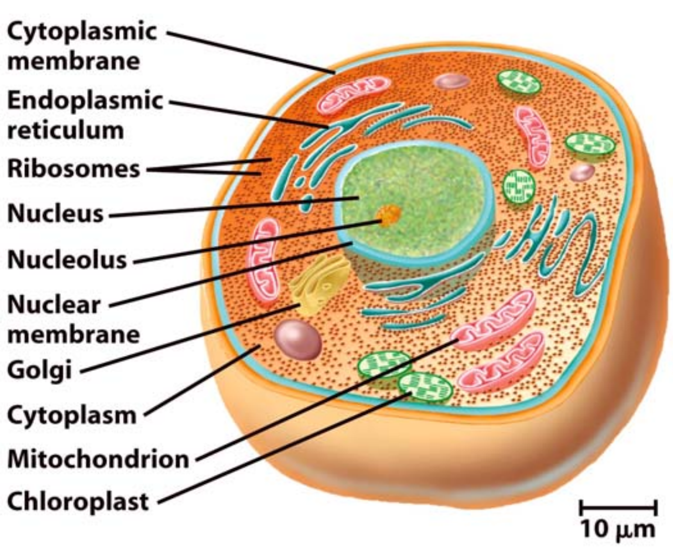
Review of some bio-basics

- Cellular organization, structure, and composition
- Macromolecular synthesis and assembly
- Biological templates for nanotechnology

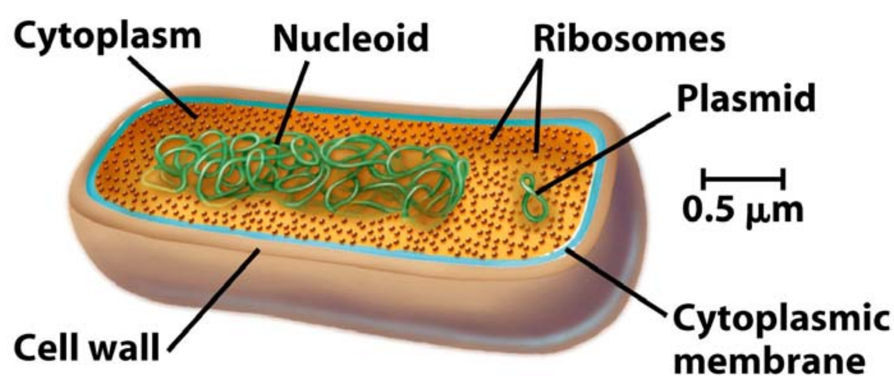
Central dogma and cellular activities



Structure of eukaryotic cell



Structure of bacterial cell



Note the refreshing absence of intracellular barriers!

The story of a bacterial cell

Do the numbers: a cell contains

- 55% protein
- 20.5% RNA
- 3% DNA
- 9% lipid
- 8.5% other macromolecules
- 3% "building blocks," metabolites
- 1% inorganic ions (Major: K, Na, P, Mg, Ca, Fe, S; minor: Zn, Se, Ni, Mo, Mn, Cu, Co, Cr, B)

The story of a bacterial cell, part II

A bacterial cell can contain:

- 70% water
- 1 copy of its chromosome
- Up to 50,000+ ribosomes (i.e, 1/4 of its mass)
- 1000+ mRNA species (each molecule typically persisting for 1-3 min)

A bacterial cell can adapt to environment by:

- Varying the spectrum of genes expressed (which of its 3000 genes transcribed)
- Varying the number of ribosomes (more ribosomes = faster growth rate)

Flow of macromolecules in a bacterial cell

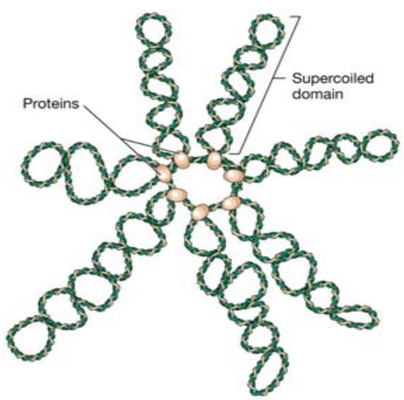
Central dogma: DNA→RNA→protein

- Similarities/differences in synthesis for these macromolecules
- How cell organizes these molecules

- How can we manipulate the system? Why should engineers, physicists, or chemists care?

Packing problems for chromosomes

All organisms must condense their DNA to fit inside the cell.



(d) Chromosomal DNA with supercoiled domains

A bacterial chromosome in linear form ≈ 1mm long. Their closed circular chromosomes are condensed and <u>supercoiled</u> by topoisomerases.

Eukaryotes do this by twisting linear DNA around histones

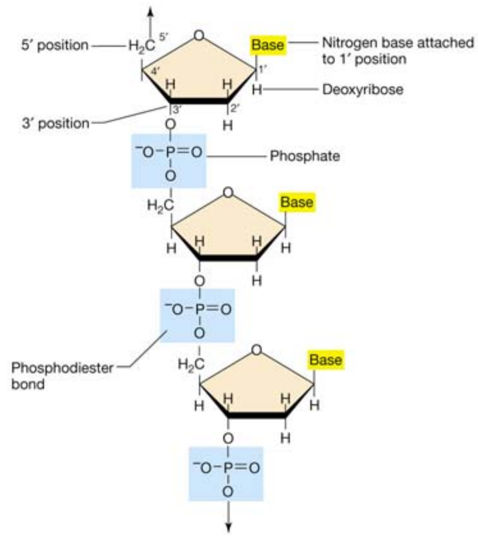
The packing of DNA affects replication, segregation and transcription.

A brief review: Basics of DNA and RNA chemistry

DNA and RNA are nucleotide polymers, joined by <u>phosphodiester</u> bonds

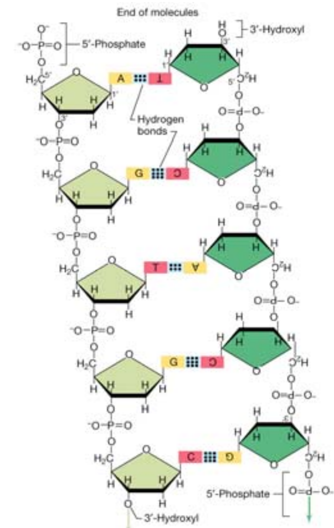
DNA with deoxyribose…

C atoms in sugar numbered with 5 and 3 C in bonds, used to describe the directionality



DNA and RNA molecules

- Cellular DNA is largely double stranded (ds) while RNA is largely ss
- dsDNA in helical conformation
- 2 strands are <u>antiparallel</u>
- 2 strands held together by <u>hydrogen bonds between the bases</u> on each strand
- <u>Specific base-pairing</u>: G-C and A-T (or A-U)

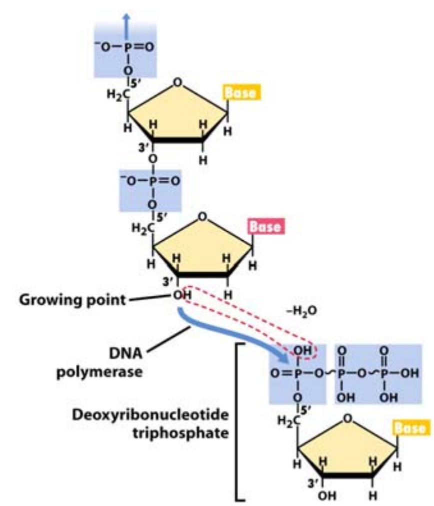


Synthesis of DNA (and RNA)

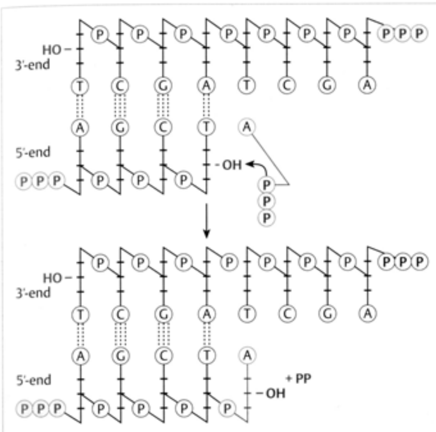
- An enzyme-mediated process, catalyzed by polymerases, using dNTPs (and rNTPs)
- Polymerization occurs 5’→3’
- Polymerases require a template
- Bacterial chromosomes generally circular dsDNA molecules, replicated from a single origin point in bidirectional process
- Eukaryotic chromosomes generally linear dsDNA molecules, replicated from multiple origins

DNA polymerase reaction

- Incoming dNTP is attached via αPi (5’) to the 3’OH of growing chain
- Pyrophosphate is released during reaction
- Energy released by cleaving Pi bond catalyzes reaction

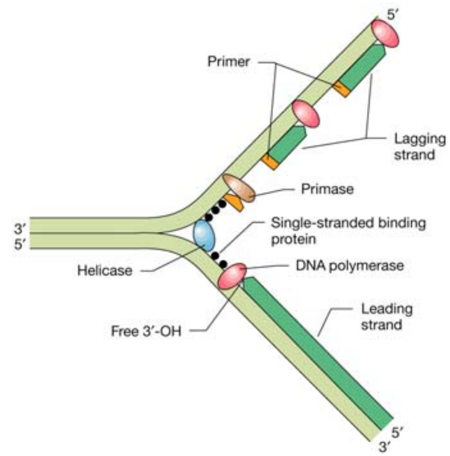


DNA polymerase reaction



- Polymerases require a template
- Base of added dNTP is complementary to template base
- 5' end with -PiPiPi and 3' end with -OH

DNA replication fork

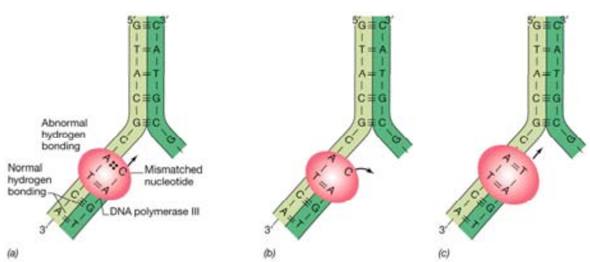


- DNA Pol is main enzyme (with 8 subunits)
- Essential roles for Primase (to start new strand synthesis) and Helicase (creates ssDNA away from *oriC*)

Efficiency of DNA polymerases

Replication is fast: 750-1000 bases/sec

Replication is accurate, partly due to fidelity of dNTP binding and to 3'→5' exonuclease activity in DNA pol that can remove mistakes

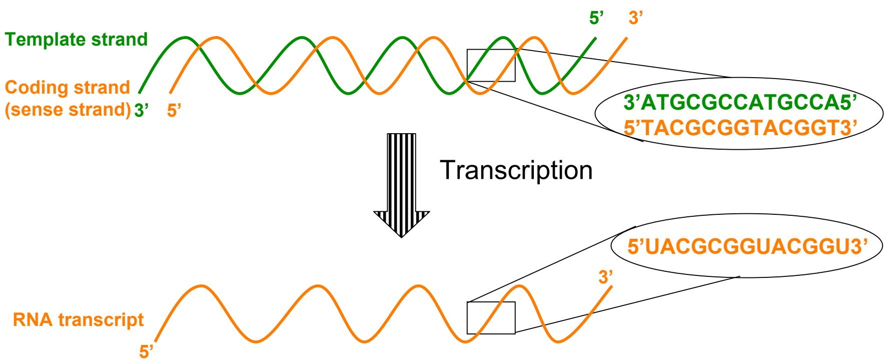


1 mistake in 10^9 nts

Synthesis of RNA in bacteria

- An enzyme-mediated process, catalyzed by a RNA polymerase (RNA pol) using DNA template and rNTPs
- Single RNA pol; primary catalytic complex is $\alpha_2\beta\beta'$ (core enzyme)
- Eukaryotes with 3 RNA pols

Overview of RNA synthesis



During DNA replication, it is critical that DNA polymerase copy all cellular DNA. In contrast, the function of RNA polymerase is to copy one strand of duplex DNA into RNA. This only occurs at particular locations on chromosome.

DNA regions transcribed into RNA

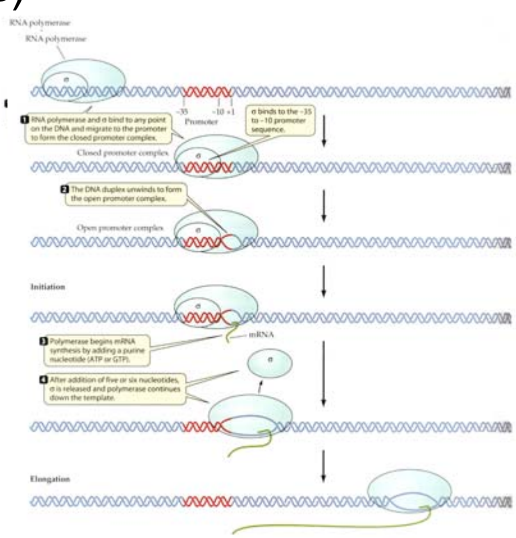
Specific regions of DNA are transcribed into RNA. These are generally called genes– specify the production of a protein (via mRNA), a tRNA, or an rRNA.

The DNA sequence that determines where transcription starts is called a promoter.

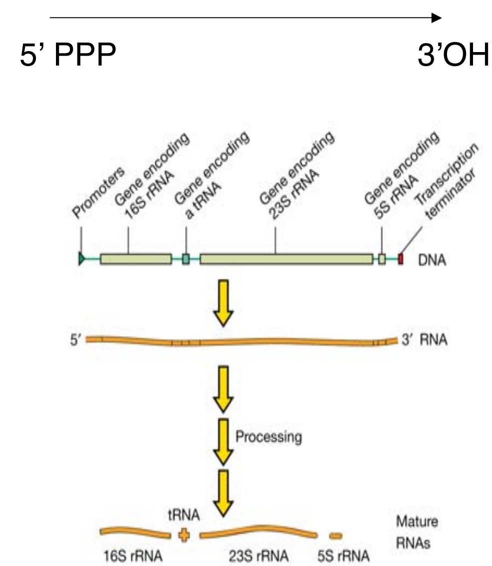
Stages in transcription (RNA synthesis)

- Initiation
- Elongation
- Termination

Process occurs without other enzymes to unwind DNA, at 20 nt/sec



Different RNA species in bacteria



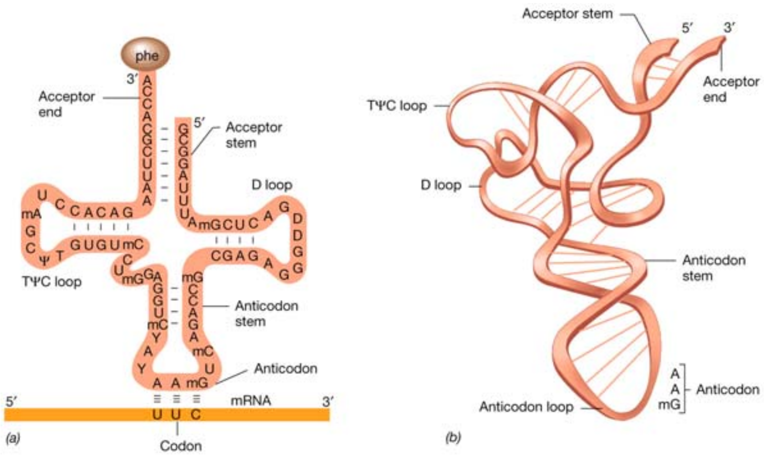
Unprocessed RNAs:

- mRNA specifies aa sequence in translation

Processed RNAs:

- rRNAs form skeleton of ribosome, catalyze peptide bond formation (23S)
- tRNAs are adaptors between ribosome and message, bringing correct aa to translation complex

Structure of tRNAs



Translating the genetic code

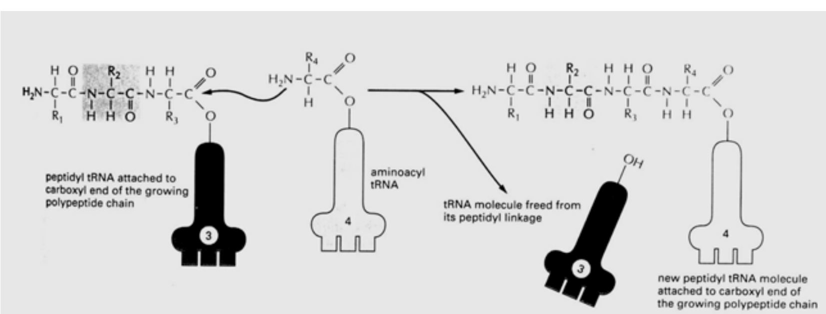
mRNA sequence decoded in 3 base sequence called the **codon**. 64 different codons.

Most codons are interpreted in same way in different organisms (universal genetic code).

- 61 codons able to specify 1 of 20 aa's, therefore most amino acids with >1 codon.
- 3 codons are not recognized by <u>any</u> tRNA: nonsense (stop) codons, UAA, UAG, UGA, that serve to tell the ribosome where polypeptide synthesis stops

The process of translation

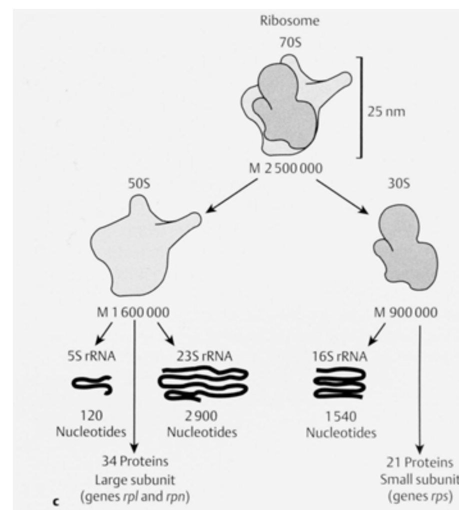
Protein synthesis: Peptide bonds form between a growing polypeptide chain and an amino acid (both attached to a tRNA)



Structure of bacterial ribosome

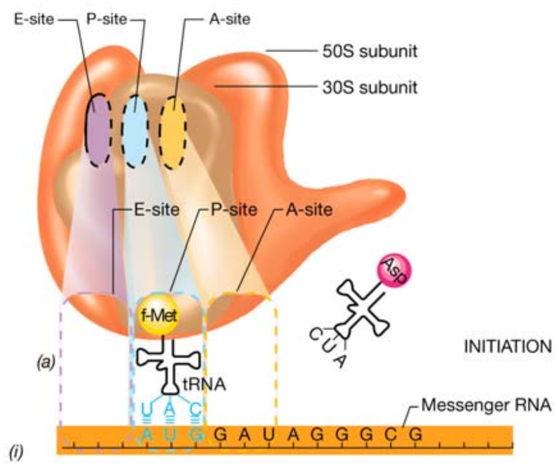
30S and 50S subunits compose 70S: intact ribosome translocates along mRNA and polymerizes polypeptides

30S subunit (with 16S rRNA) critical for selecting translation start site



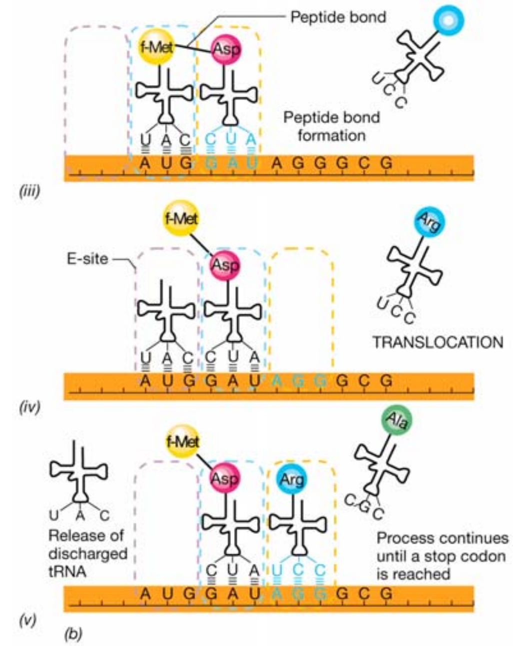
Translation and the ribosome

- tRNAs in 3 sites: A, P, E
- Polypeptide chain on tRNA in P site (peptidyl site)
- Charged tRNA with aa in A site (acceptor site)



Translation

- Peptide bond forms between COOH on tRNA in P site (peptidyl site) and NH_3 group of aa in A (acceptor) site
- Transient attachment of peptide to tRNA in A site, then translocation of ribosome
- P site tRNA to E (exit) site
- Synthesis proceeds: NH_3 to COOH
- Overall rate in mesophilic bacteria about 20 aa/sec



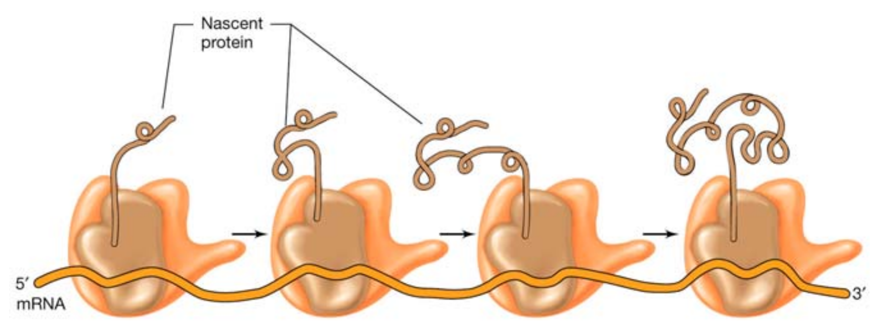
Protein folding and movement in the bacterial cell

- All protein synthesis occurs in cytoplasm
- Generally, product of translation is unfolded polypeptide
- Polypeptides have to fold into proper 3 dimensional structure in order to function
- 20% of polypeptides synthesized in the cell ultimately are localized to the cytoplasmic membrane or are translocated to extra-cytoplasmic compartment

Newly synthesized polypeptides in cytoplasm

Will typically see multiple ribosomes/mRNA.

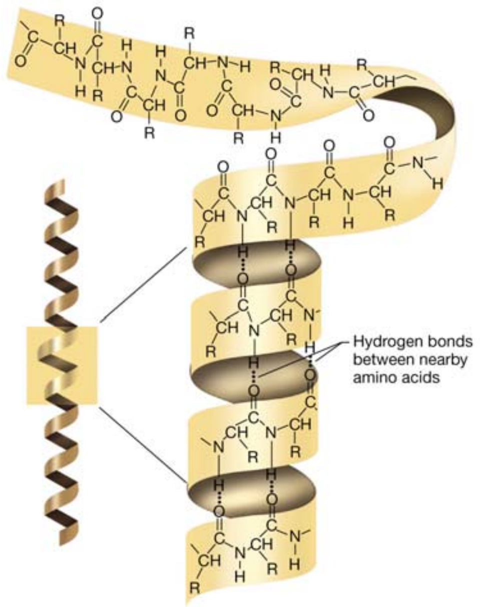
Peptide folding <u>often</u> will start before translation is complete (but not always). Primary aa sequence will largely determine secondary structure.



Secondary structural elements of proteins

Basic types of protein 2° structure, due to H bonding between residues in peptide backbone

- α helix (shown)
- β strand

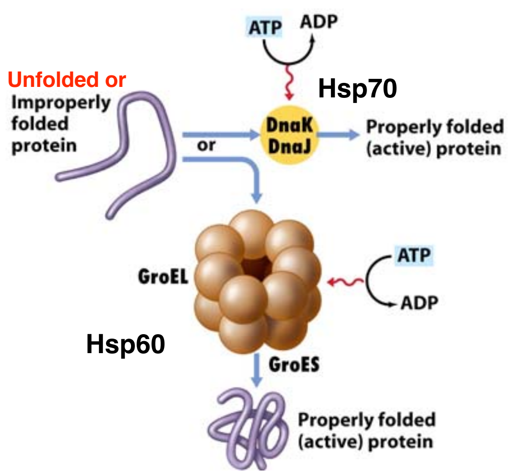


Folding of newly synthesized polypeptides

- Secondary protein structures(alpha helices and beta strands) often form spontaneously
- Tertiary/Quaternary protein folding can occur spontaneously but frequently is aided by molecular chaperones
- Chaperones can interact with newly synthesized (and unfolded) or with misfolded polypeptides
- Chaperones can help polypeptide attain structure, but are not part of the final protein structure.

The action of chaperones

Chaperones interacting with misfolded or unfolded cytoplasmic proteins often hydrolyze ATP during proper folding

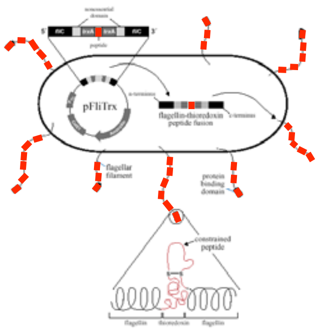


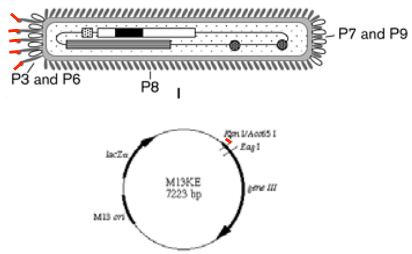
Manipulation of proteins for fun and nanotechnology

A fully folded protein often has significant surface area, exposed to aqueous environment. These surfaces can sometimes/often be derivatized/engineered (ideally without compromising the nature of protein):

- Can functionalize reactive groups via chemical means (e.g., reactions with thiol or amino groups)
- Can engineer the coding sequence of the gene to include novel polypeptide sequences in the polypeptide during translation, which will end up on the surface of the protein after folding

Display technologies: FliTrx and PhD





- **Principle: cell surface display within the active site loop of TrxA which is itself inserted within FliC**
- **Flagella copies per cell: 10-15**
- **Random segment size: 12 aa**

- **Principle: display at the N-terminus of phage M13 P3 protein**
  P3 copies per virion: 5
- **Random segment size: 7 or 12 aa**

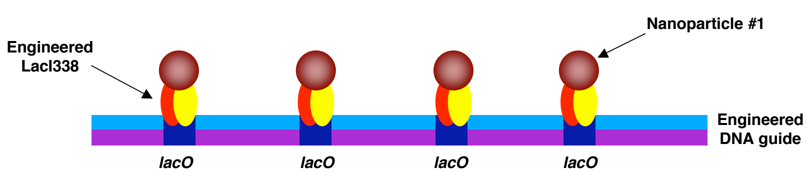
Can use display libraries to identify polypeptide sequences with novel and interesting properties.

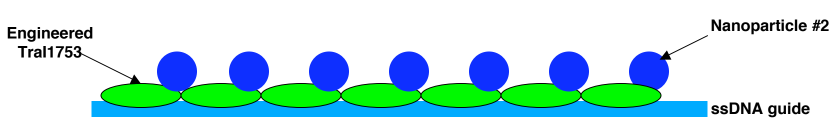
Using peptides for nanotech

After identification of peptides sequences with interesting characteristics, a resourceful nanotechnologist can start to have fun!

If peptides have desired properties in different protein contexts, can move sequence from gene of original isolation into other suitable platforms.

Application: Engineer DNA-binding proteins for organizing inorganic compounds bound to them



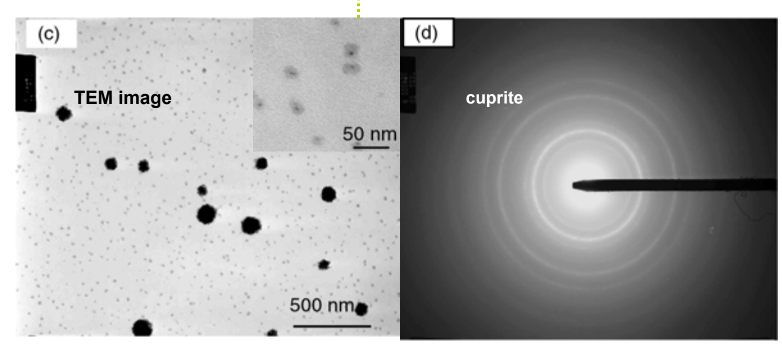


Methodology: move coding sequence for inorganic-binding peptide (from library) into gene for DNA-binding protein: what does engineered protein do?

Formation of Cu_2O particles

Can form Cu_2O from $CuCl_n^{1-n}$ at pH 12.

At pH 8, TraI::CN225 Cu_2O binding motif can lead to formation of appropriate nanoparticles, with protein conc. controlling particle size



Cu_2O particles form from $CuCl_n^{1-n}$ after addition of TraI1753::Cu_2O motif (2.4×10^{-8} M protein). (Dai, Choe, Traxler, Baneyx, & Schwartz)

Arrangement of particles

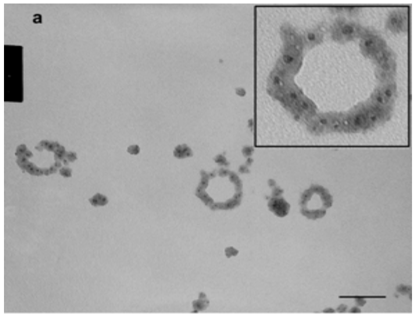


Figure 3. TEM image of TraI::CN225-DNA-NanoCu_2O assembly. The bar represents 100 nm

TraI1753::CN225 can lead to the organization of Cu_2O nanoparticles onto a DNA substrate (provided here by φX174 DNA)