Lecture 22
4/7/04

The next exam will be wed 4/14 from 7:30-9:30pm

Look at the rasmol scripts on the 5.08 website under resources

G-proteins

Look at p. 4 of handout 3b for a cartoon overview of G proteins

Remember that the GTPase domain is highly conserved. This domain is also conserved in Motor proteins (use ATP) like myosin and kinesin see also p. 4 of handout 3b. These types of molecular motors accomplish diverse functions like muscle contraction and chromosome separation during mitosis, movement of organelles.

GTPase:

1. Conserved 21 kDa domain, 5 helices, 6 strands (5 parallel, 1 anti-parallel)
2. common P loop (to GTPases and ATPases)
   $GX_4GK(T/S)$
   Conserved flexible glycines in nucleotide binding sites- also K binds alpha, beta phosphates of GTP
3. Switch I (usually colored green)
   -----T----
   one conserved threonine, -OH interacts with Mg^{2+} and gamma-phosphate of GTP
4. Switch II
   -DXXG
   flexible glycine, H-bonds using its backbone NH of amide
   conserved aspartate interacts with metal Mg^{2+}

See page 4 of handout for examples of structures of similar G domains in G proteins and motor proteins (use ATP)
What we learn about G proteins will help you think about a large variety of other proteins and reactions

See p 7 of handout for a comparison of GTP form and GDP form of EF-Tu

What the switches do depends on what the effector is

G proteins have distinct structures in the GTP bound state and the GDP bound state

GTP Bound: Tight binding to effector, tight binding of nucleotide, but very low GTPase turnover

GDP Bound: no binding effector (conformational change releases effector), tight binding of nucleotide

3 regulatory mechanisms:

a. GAPs = GTPase Activating Partner (usually a protein, can be RNA) accelerates GTPase actvitity 10^3-10^5 fold
b. NEFs= nucleotide exchange factors (also GEFs, guanine), usually proteins, allow GDP to be replaced by GTP
c. Small protein inhibitors also bind GTPases ex. Ras (not pertinent to EF-Tu)

Ef-Tu delivers charged tRNA to the ribosome
Fidelity??

Structure: anticodon/codon interaction?
Insight from structures of Rama Krishnan structure of the 30S ribosome subunit
Key player is 16S RNA in the 30S subunit

Models derived from antibiotic studies

1. paromycin
2. streptomycin

both cause miscoding (insertion of the incorrect amino acid)

conformational changes of tRNA upon binding, we've talked about the comparison of cryoEM and Xray structures
fluorescence methods can also be used to look at these conformational changes

EF-Tu GTP tRNA → via GAP (dramatic conformational change) EF-Tu GDP → via EF-Ts (NEF) dissociates GDP, picks up GTP → EF-Tu GTP tRNA

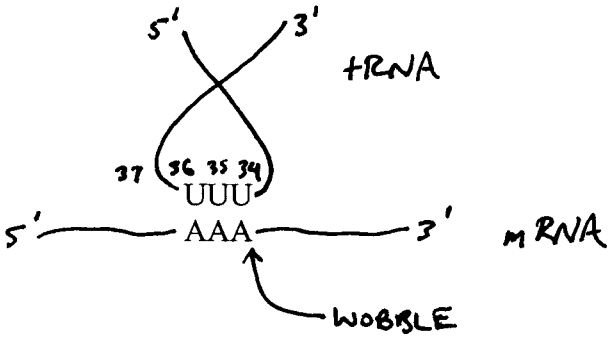
p. 3 of handout shows structural pictures that have helped us understand cognate, near-cognate, and non-cognate interactions.
Important:
Helix 44, adenines 1492, 1493
Helix 18, loop guanine 530
These residues all interact right in the region of the codon/anticodon interaction,
As do both antibiotics
These experiments use a small stem-loop structure in place of the tRNA in the Asite

Look at the base pairing and the role of the G530, A1492, A1493 in the active site

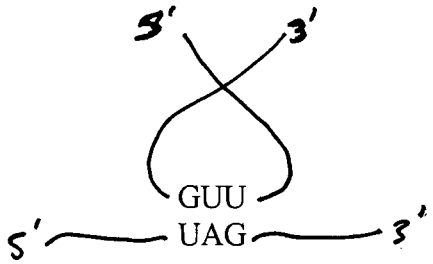
Structure gives us a model of how we distinguish between cognate, near-cognate, and non-cognate interactions.
Gives us a basis for conformational changes

Ef-Tu (fidelity)
How do we distinguish between cognate, near-cognate, and non-cognate interactions?
All DNA/RNA polymerases must be able to sense the correct bp interactions as well.
H-bonding interaction is insufficient to explain this discrimination in both cases

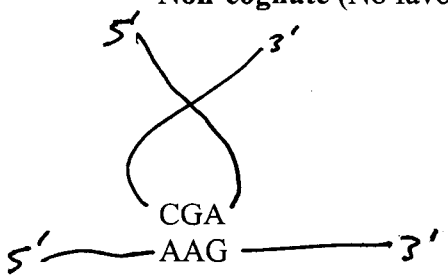
Cognate (1st 2 base pairs are correctly H-bonded, 3rd position is the wobble position, flexible, can be anything)



Near-Cognate (1 correct base pair in first two spots)



Non-cognate (No favorable H-bond interactions)



Remember codon/anticodon interaction must be anti-parallel

Key Question:
Are the H-Bond interactions between base pairs sufficient for fidelity?
Answer: No!
How does Ef-Tu work? What is the role of GTP in enhancing fidelity??

See page 8 of handout 3b for a picture of the cartoon model for EF-Tu fidelity

Initial binding → noncognate interaction → dissociation of
EF-Tu•GTP•tRNA (discriminate from non-cognate without using GTP

OR

Initial binding(of Ef-Tu•GTP•tRNA to the ribosome)→ codon recognition = tighter binding → GTPase activation

RNA is the GAP!
Makes GTP hydrolysis fast
(rapid irreversible chemical step)
→ EF-TuGDP dissociates

Next step is ether accommodation or proofreading

If a cognate interaction, accommodation occurs accompanied by a conformational change, charged tRNA leans over to Psite so that a peptide bond can form. The peptide bond formation is rapid and irreversible and drives the reaction to the right once the correct positioning occurs

Or if near cognate, proofreading, tRNA dissociates

EF-Tu: selection before GTPase (GTP hydrolysis) AND proofreading after hydrolysis