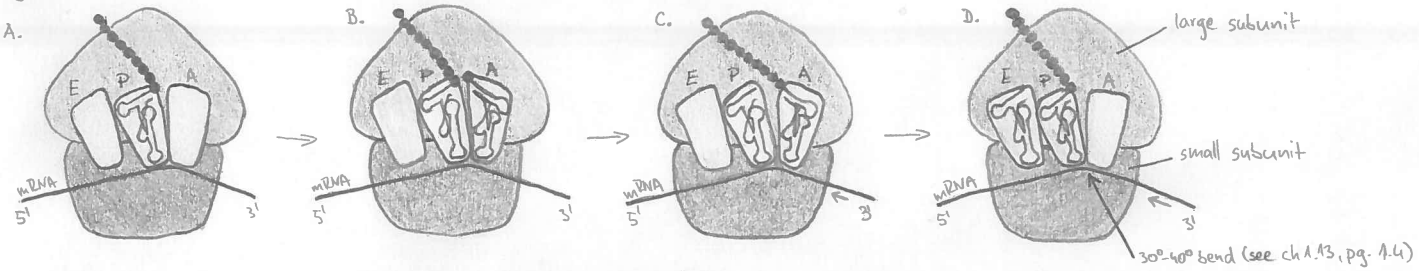
2. Translation II

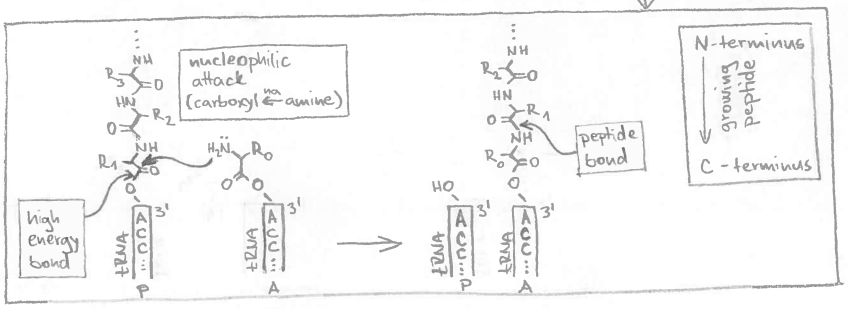
2.1 Phases of Tnl

- initiation: assemble ribosome at the initiator codon (usually AUG), such that it can start forming peptide bonds and extending polypeptide chain
- elongation: add amino acids one by one, until the complete polypeptide is formed
- termination: release
  - peptide from the tRNA it is bound to
  - ribosome from the mRNA

- elongation



recruit charged tRNA into A-site → form peptide bond (peptidyl transferase reaction) → translocation



- growing peptide is always attached to tRNA
  - either P-tRNA (A,B,D), or A-tRNA (C)
- tRNA that is attached to growing peptide is safely embedded inside the ribosome
  - anticodon-mRNA interactions
  - tRNA-ribosome interactions
- no ATP used in forming peptide bond, energy from ATP hydrolyzed during tRNA charging (stored in high energy bond: A-aa) is used instead

2.2. GTP-Regulated Auxiliary Tnl Factors

- change conformation based on nucleotide bound (GTP vs. GDP vs. GDP+P_i)
- change in conformation ⇒ change in affinity for tnl components
  - typically
    - GTP bound: ↑ affinity for target
    - GDP bound: ↓ affinity for target

  ⇐ e.g. both EF-Tu and EF-G bind to the A site (regulated by GTP→GDP hydrolysis)

- all events in tnl use GTP-regulated factors to ensure
  - execution in the correct order
  - fidelity
  - number of factors
    - initiation: 2 (eukaryotes), 1 (bacteria)
    - elongation: 2
    - termination: 1

2.3. Structural Characteristics of EF-Tu

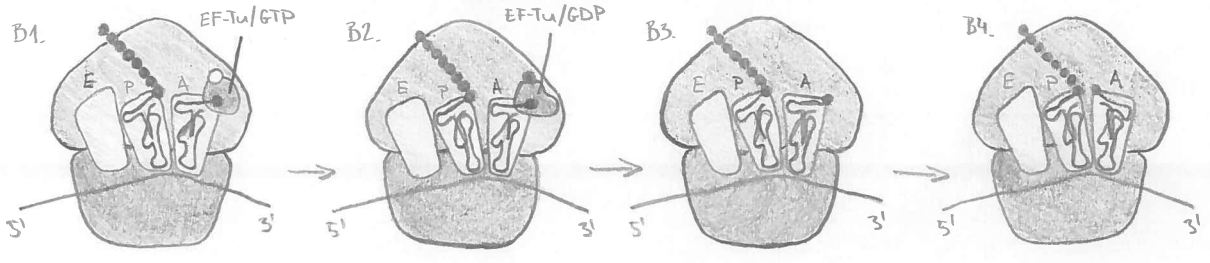
- bacteria: EF-Tu
- eukaryotes: eEF1 (Eukaryotic Elongation Factor 1)

} both work in virtually the same way

- delivers aminoacylated (charged) tRNA to the ribosome
- binds tRNA
  - GTP-bound state: YES
  - GDP-bound state: NO

} significant change in conformation ⇐ only aminoacylated (charged) tRNAs are bound

- protects aminoacylated end of tRNA (containing the high-energy bond: see ch. 2.1) by making it inaccessible ("burried" inside the protein structure)
  - catalysis can not occur, while charged tRNA is bound to EF-Tu/GTP



EF-Tu delivers charged tRNA to the A site

correct codon-anticodon pairing allows the factor binding center (sarcin-ricin loop) to stimulate GTP hydrolysis

GDP-bound EF-Tu dissociates from the ribosome due to low affinity to aa-tRNA

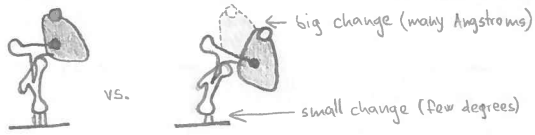
A-aa-tRNA undergoes accommodation to position the aa for peptide bond formation

2.4. EF-Tu and Accommodation

- GTP-bound ET-Tu w/ aa-tRNA comes in and samples the anticodon
- if the correct codon-anticodon interaction occurs, it triggers additional changes
  - interactions of codon-anticodon bp w/ small ribosomal subunit (30S in bacteria) ← numbering of bases is different in euk., but they are present and equally positioned in all organisms
    - A1492, A1493 (hydrogen bonds w/ minor groove of codon-anticodon bps) → flip out of their normal position
      - form bp-nonspecific interactions w/ the 1st (A1493) and 2nd (A1492) base of the codon
      - similar to e.g. DNA Pol recognising (bp-nonspecific) base pairing
    - G530 — rotates from syn- to anti- position (rotates around the axis defined by ribose-guanine bond)
      - interacts w/ wobble base, minor groove of the 2nd bp of the codon (to some extent) } interactions w/ 2' OH ← the extra OH present only in RNA (not DNA) (w/ codon in one case, w/ anticodon in the other case)
  - distortion of aa-tRNA
  - EF-Tu factor binding center (sarcin-ricin loop)
    - His84 — rotates out of original position to interact w/ GTP; allows GTP hydrolysis to occur (the His is required for catalysis) ← IRREVERSIBLE STEP: selection ⇒ proofreading
  - EF-Tu changes conformation (GTP-bound vs. GDP bound) and is released from the ribosome/aa-tRNA
  - A-aa-tRNA undergoes accommodation (see B3 ⇒ B4, ch. 2.3, pg 2.1)
    - the acceptor arm turns around to bring aa close to nascent peptide strand in order to allow formation of peptide bond
- there are 61 possible codons/anticodons and only single one of them is correct (translation: 1 of 61 vs. replication/transcription: 1 of 4)
  - the above mechanism is triggered only in presence of correct codon
  - one of the reasons why translation is much slower than replication/transcription

2.5 Fidelity of Correct Codon-Anti-Codon Pairing

- EF-Tu GTP hydrolysis dramatically stimulated by correct codon-anticodon interaction
  - even a small change in base pairing will cause very big change in positioning of tRNA acceptor arm and GTP



for hydrolysis ↓

  - there are conformational changes propagated through tRNA on correct codon-anticodon basepairing, which are needed for correct positioning of GTP
- accomodation places strain on codon-anticodon interaction
  - acceptor arm of aa-tRNA has to rotate ~60° to allow peptide bond formation

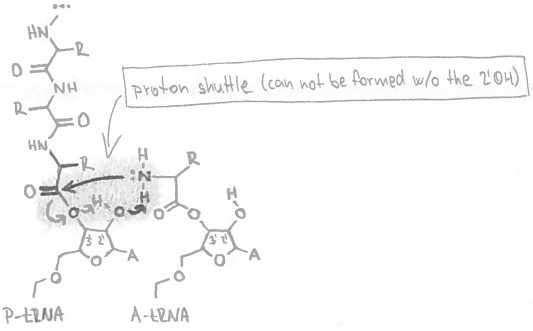
(see B3 ⇒ B4, ch 2.3, pg 2.1)

the difference can be as little as 1-2 hydrogen bonds →

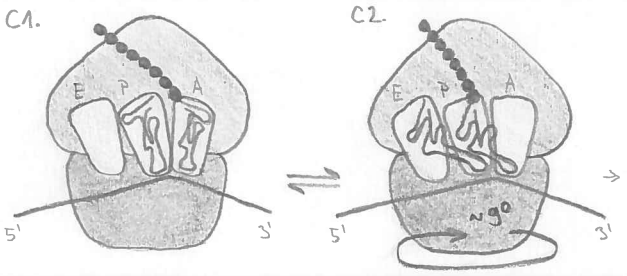
  - correct codon-anticodon pairing will resist the strain
  - incorrect codon-anticodon pairing has high probability of breaking ⇒ release of incorrect aa-tRNA before formation of peptide bond
- EF-Tu seems to have sort of proof reading capacity: its affinity for incorrectly charged tRNAs seems to be lower ← there is strong evidence, but this has not been prooven
  - Ala-$tRNA^{Cys}$ (similar aa substitution): EF-Tu will bind
  - Trp-$tRNA^{Cys}$ (dissimilar aa substitution): EF-Tu will NOT bind
  - this seems to be caused by combination of tRNA-TF-Tu interactions, aa-TF-Tu interactions } "opening", where aa fits into TF-Tu prefers hydrophobic interactions and tRNAs for hydrophobic amino acids have slightly smaller affinity to TF-Tu
  - papers by Uhlenbeck

2.6 Mechanism of Peptide Bond Formation

- ribosome
  - no protein anywhere near the site of peptide bond formation (nearest one ~25Å away) ⇒ strictly catalysed by RNA
  - many conserved bases around the active site: but mutating them will only slightly slow the reaction
  - entropic catalysis: positions everything at the right place (3' ends of A-tRNA and P-tRNA are very close to one another)
- tRNA — 2' OH of the 3'-most adenine of tRNA is the critical catalytic residue driving the reaction (reaction virtually stops w/o this 2' OH)



2.7. TRANSLOCATION



C3. GTP → C4. GDP+Pi → C5. GDP → D. ch.2.1 pg.2.1

classic state ⇌ hybrid state

(oscillation)

"Brownian ratchet"

can be monitored using FRET pair b/w 30S and 50S subunits

[EF-G (bacteria) / eEFII (eukaryots)]

- GTP-bound form: binds and stabilises the hybrid state (C3.)
- GTP → GDP + Pi hydrolysis (C4.) ← driven by A2662 in sarcin-ricin loop
  - undergoes conformational change
  - unlocks the barrier b/w P/A sites, preventing anticodon loops from translocation
    - this barrier probably contributes also to mRNA bending
  - allows mRNA to be shifted (by 3nt) by translocating tRNA (EF-G will occupy the A site, tRNAs P and E sites)
- Pi is released (C5.)
  - undergoes another conformational change
  - EF-G is released from the A site of the ribosome

part of EF-G occupying the A site mimics shape of tRNA+EF-Tu

2.8 ENERGETICS AND EF-G

- single peptide bond formation (vs. 1 ATP per incorporated nt in replication/transcription)
  - ATP: coupling of AA and tRNA
  - GTP: aa-tRNA delivery
  - GTP: translocation

ATP: (indirectly) provides energy needed to drive peptide bond formation reaction

GTP: control order of steps and specificity (not directly contributing to ↑)

- EF-Tu hydrolyzes GTP to allow peptide bond formation
  - EF-Tu-aatRNA can now "accommodate"
  - aa is physically blocked by EF-Tu
  - also contributes to specificity
- EF-G/GTP can bind only if large subunit A site is empty (hybrid conformation)
  - peptide bond has been formed
  - EF-Tu is no longer present (it would physically block EF-G from binding)
- EF-G/GDP+Pi drives translocation
- EF-G/GDP is released after translocation
  - only after its release can EF-Tu bind (it would physically block EF-Tu from binding)