RNA Structure

- Structural, Catalytic, Regulatory
- Ribose-Unstable Backbone, Uracil-Deaminated C, Single stranded
- Thermosensor in pfrA activates transcription in high temp, trp only translated in low trp levels, Gag-pol of MLV translated mostly Gag, 5-10% Gag-Pol via pseudoknot formation

mRNA

- ORF(start, stop), Prok → RBA/Shine-Dalgarno-16S rRNA Euk. → 5' M-Guanine cap, Scanning, Kozak-initiator tRNA
- 5' Cap first: 7-M-Guanine attached backwards, 5'-5' phosphate link, cytosol by methyltransferase
- Poly-A: 40-200 to 3' from ATP, polyA signal → AAUAAA cleaved, poly-A added to new 3', nuclear exit depends on it

tRNA

- Adaptor, 75-95 nt, 3' CCA conserved, Pseudo uridine, dihydrouridine(D), hypoxanthine, T, m-G all improve tRNA function
- Acceptor: codon attached to 3', pseudo-U loop, D loop, Anticodon Loop (3' always purine, 5' always uracil), Variable loop
- L-Shaped 3 structure, acceptor 70A from anticodon loop
- Stabilized by: Base stacking in extended regions, unconventional H bonds-different helixes, base-sugar/Pho backbone

rRNA

- Structural and catalytic role, peptidyl transferase and decoding center → entirely rRNA, proteins peripheral

Splicing

- 5' : GU, 3': AG, Branch: A, pytract: pyrimidines following branch, all within intron
- 2'-OH at branch attacks phosphoryl of G as 5' site, 3-way junction. -OH of 5' exon attacks 3' site, releasing lariat
- No gain of chemical bonds, 2 broken 2 form

Spliceosome splicing

- 150 proteins, 5 RNAs: U1, U2, U4, U5, U6 and non-snRNPs are U2AF (pytract) and BBP (Branch)
- E: U1-5' site, BBP-branch, U2AF-3' site and pytract
- A: U2-Branch(aided by U2AF displaces BBP and forms bulge at A
- B: U4, U5, U6 (tri-snRNP particulate) bring together all 3 sites
- C: U1 leaves, U6-5' site, assembly complete, U4 leaves, allowing U6 and U2 to interact
- C complex creates active site with 5' site to branch for 1st transesterification, 2nd aided by U5 bringing exons together
- Irreversible because: DEAD-BOX helicase(Spliceosome) , Lariat degraded

Other splicing

- Minor spliceosome → different recognition sites, U11 and U12 instead of U1 and U2
- Group-I → smaller than II, conserved 2nd structure containing IGS → binds 5' site, 3rd structure → extra G, linear
- Group-II → same reaction pathway

Splicing Control

- Errors: Splice site skipped, Pseudo-site recognized usually 3' exon cut early
- Accuracy: 1) co-transcriptional loading of splicing proteins, SR proteins bind to exonic splicing enhancers(ESE)-U1/U2AF
- SR essential: 1) Increase accuracy 2) regulate alternative splicing 3) Cell specific splicing
- Trans splicing: Different mRNA spliced together, intron released as Y shape, exon-exon is the same

Alternative Splicing

- Five ways: Normal, Exon skipped, exon extended, intron retained, alternative exon order
- Regulated by exonic/intronic enhancers/silencer, isoforms
- SR proteins: RRM and RS(SR-spliceosome interaction) domains
- hnRNPs: Silencers, RRM but no RS

Exon Shuffling

- Intron early, Intron late, retained in Euk. Because: 1) Increased proteome 2) reshuffled by functional units
- Proof: 1) Exon-intron border → domains 2) Duplication and divergence 3) Related exons in unrelated genes

RNA Editing

- Post translational, individual bases, RBB for site specific
- Site specific Deamination C→U, early stop UAA from CAA

- ADAR: A→I, altered Ca2+ channel, needed for brain development
- gRNA: Inserts U into mRNA correcting ORF, anchor-editing-polyU site

Translation

- Carboxyl of AA linked to 2' or 3' -OH of terminal A, Acyl linkage: 1) Adenylation (AMP) 2) tRNA charging
- Class-I tRNA synthases attach 2'-OH, monomeric Class-II attach 3'-OH, di or trimeric
- 20 tRNA synthases common, specificity: Acceptor stem, anticodon loop. Discriminator base in acceptor most important
- Editing pocket → valine vs isoleucine

Prokaryotic Initiation

- Alignment of small-RNA → RBS and 16S RNA, P above start codon
- fMet → prokaryotic initiator -structural resemblance to peptidyl-tRNA for P site,
- Deformylase removes formyl from fMet, aminopeptidase removes methionine +others which might inhibit folding
- IF3 binds E of small-prevents large reassociation, IF2gtp and IF1 bind A- facilitate initiator binding
- With all 3 IF, small binds RBS, initiator enters P binding AUG → IF3 release → large binds → IF2gdp → IF2/3 leave → 70S

Eukaryotic Initiation

- Small recruited to 5' Cap, small + initiator + factors scan until AUG is found
- eIF1, eIF3, eIF5 bind E, prevent large reassociation, eIF1A binds A, eIF2-gtp escorts initiator to P, formed without mRNA
- mRNA prepared separately: eIF4E binds 5' Cap, eIF4G bridges A-E, eIF4A helicase activity, eIF4B activates A, 43s complex
- 43s Complex resolves 2nd structures in mRNA, association between factors brings mRNA to small, 48S formed
- Anticodon-codon base pairing stimulates eIF2gdp → eIF2, eIF1, eIF3 and eIF5 released
- eIF5B recruits large → releasing eIF5Bgdp and eIF1A, 80s formed
- eIF4G binds poly-A-binding proteins in tail, circularizing mRNA promoting efficient translation

Elongation (Prokaryotic, general)

- EF-Tu-gtp escorts aminoacyl-tRNA to A site → EF-Tu-gdp disassociates by GTPase activation in factor binding center
- Ribosome selects: 1) 2 A residues in 16s rRNA in A 2) EF-Tu GTPase depends on favorable geometry 3) Accommodation
- Peptidyl transferase center in large → 23S, as is L27 (not essential), 23s and 3' CCA of tRNA brings substrates of P-E close
- Peptidyl transferase → substrate assisted, 2' -OH of P acts as proton shuttle to the 3' -OH
- 3' -OH acceps proton from NH2 of A site tRNA, energy comes from acyl bond in charging
- EF-G: A→P→E by binding A and factor binding center, hydrolyzing GTP allows translocation, moves ribosome forward
- Elongation requires 2 GTP 1 ATP for each AA added, plus 1 GTP for initiation (eIF2 and IF2)

Termination (Prokaryotic, general)

- Class-I RFs recognize stop codon, activate hydrolysis: RF1 for UAG/UAA and RF2 for UGA/UAA
- Class-II RFs remove class I RF: RF3 and eRF3.
- RF3-gdp binds ribosome → exchanges for GTP → kicks out RF1/2, hydrolyses GTP to leave
- Ribosome Recycling Factor mimics tRNA: with EF-Tu releases P tRNA directly, with IF3 binds E site blocking reassembly

Regulation

- Mainly at initiation, rapid response vs transcriptional control, blocking the RBS via: RNA binding proteins or 2nd structures
- Ribosomal proteins in operons which inhibit their own translation, mRNA increase but protein doesn't → auto inhibition
- rRNA and mRNA have similar 2nd, ribosomal prot prefer rRNA, but if non is available bind RBS of mRNA for themselves
- Ferritin mRNA: IRP without Fe binds IRE, stabilizing 2nd → no ferritin, while IRP-Fe cannot bind IRE, 2nd resolved → ferritin

Genetic Code

- Degeneracy, many codons same AA, usually U-C and G-A, limits delirious effects of mutations
- Inosine: anticodon base from deaminated A
- Wobble: 5' anticodon base is less sterically confined, G:U/C, C:G, A:U, U:A/G, I:A/U/G
- Three rules: 1)codons read 5'→3' 2) codons non-overlapping, no gaps 3) Fixed reading frame, set by initiator codon
- Point Mutations: Missense, nonsense, frameshift or silent
- Suppressors: Direct reversal, or suppressor – Intergenic(same) or Intergenic(different, often involve tRNA mutations)
- Nearly universal, mitochondria UGA → tryptophan instead of stop, 22 tRNA in mammalian mitochondria

Reporter genes

- cDNA → RNA: 1) Anneal primers 2) Reverse transcriptase 3) mRNA degraded 4) cDNA created as plasmid, phage vector
- Nitrocellulose paper, radioactive DNA probes mark colonies
- Reporters should: Easily detectable, low to zero endogenous expression, fused to promoter of desired gene
- B-glucuronidase(**Gus**): hydrolyses B-glucuronides, which are oxidized to form blue dye, only active in substrate rich tissues
- GFP: cyclization of Ser-Tyr-Gly, changing Tyr66 changes color
- Agrobacterium Tumefaciens integrates its plasmid DNA randomly into plant host → tDNA genes produce tumors
- Ti-Plasmid replaces oncogene genes with T-region, inserting genes on interest
- Binary vector has: 1) modified pTi containing only plasmid transfer genes 2) plasmid with left/right border, slectable marker, and polylinker for insertion of foreign gene

Gene cloning

- Goal: clone enough copies of a gene to manipulate, all identical, by forming vectors made of gene + recombinant DNA
- Restriction enzymes: cut specific sequences, cutting gene and host with same RE → sticky ends anneal, recombinant
- Three classes: 1) 5' sticky overhang (BAMH1), 2) 3' sticky (KPNL), 3) blunt (SMA1), protect bacteria from viral infection
- Cloning vectors must: 1) Origin of Replication recognized by host 2) Selectable marker 3) single cleavage site for each RE
- Vectors can be: plasmids(up to 15kb), phage (90kb) or artificial chromosome (100-2500kb)
- Events: 1) RE added to cut vectors 2) DNA fragments cut by same RE added with gene 3) Ligase forms recombinant DNA 4) Selective agent added (E.g. Penicillin) so only colonies with recombinant DNA survive
- 5' → blunt end via Klenov or T4 polymerase filling the gap
- 3' → blunt via T4 polymerase exonuclease activated by excess dNTPs, cutting back the sticky end
- Directional cloning: using two RE to cut both DNA fragment and host DNA in the same orientation
- Steps: 1) Choose RE 2) Isolate specific fragments 3) ligate vector 4) transform to host 5) grow colonies 6) Isolate plasmid DNA 7) Cut with RE to confirm foreign DNA presence 8) Run in gel electrophoresis to identify recombinant plasmid

Tissue Specific Expression

- Goal: understand role of gene by studying where and when its expressed

UAS/Gal4 transactivation:

- MPR-Gal4-UAS-GFT inserted randomly by agrobacterium, when in front on driver promoter GFP expressed
- Some recombinant progeny will express tissue specific patterns, cross with UAS-GENE-X cassette containing plant
- In those tissues we see GFP, progeny will also express gene-x due to UAS transactivation by Gal4

Cre-Lox

- Cre removes region between Lox-Lox, Cre-Lox used to study single cells with Gal4/UAS system
- Two cassettes: 1) Ubiquitous promoter-Lox---Lox-Gal-UAS-GFP 2) Heat Shock Promoter-CRE
- Distance between Lox is too long to express GFP cassette, Heat → Cre → Lox-Lox → GFP expressed
- Gene-x can be added after GFP so …GFP-UAF-Gene-x
- Cre-Lox can be used on mutants for a gene, by restoring function via Lox-genex-Lox, then heating single cells to remove
- Restored function mutants will express GFP, all others wont