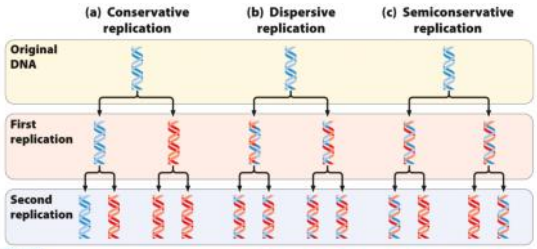
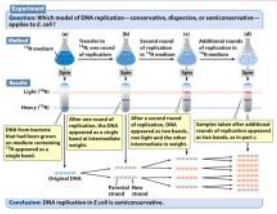
Exam 2 Material

Thursday, February 20, 2020 11:01 AM

Lecture 7 Material
DNA Replication and Repair

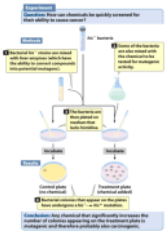
- General structure of DNA:
  - Watson and Crick base pairing - A bonds to T (U on RNA) and G bonds to C
  - A - T bonds have two hydrogen bonds, while G - C bonds have three hydrogen bonds
  - 5' end is a 5' carbon attached to phosphate group, while the 3' end is a 3' carbon bound to an OH group.
- Models of replication for DNA -
  - Semiconservative replication model - The original strands of DNA replicates, and in the first replication there is one strand from the original strand and one new strand of DNA.
    - Confirmed to be the model by which DNA replicates by Meselson and Stahl's experiment
  - Conservative replication model - in the first replication, there is a completely new strand replicated from the original strand, and the same DNA strands as before.
  - Dispersive replication - Each replication is a mixture, or hybrid, of different generations of the replicant.
- Meselson and Stahl's experiment
  - E coli were grown in 15N (Nitrogen-15) media, then transferred to 14N media. The cultured E coli DNA then underwent centrifugation
  - In the centrifuge, the heavier 15N would sit at the bottom while the lighter 14N DNA would sit closer to the top. Some of the DNA would sit between the 15N and 14N sites, as a result of the original 15N and 14N strands forming DNA that contained both.
- The semiconservative model of DNA replication
  - In E coli, there are two modes of semiconservative replication:
    - Theta replication - circular DNA, E. coli; single origin of replication forming a replication fork. Includes bidirectional replication.
    - Rolling circle replication - single origin of replication by RepA protein "nicking" the strand of DNA, allowing for the polymerase to attach to the 3' OH end on the DNA. This mode of replication is unidirectional. The 5' end will elongate out of the circle to result in a strand of circular DNA.
  - Linear eukaryotic replication
    - Eukaryotes can have thousands of origin sites (ORI) in their DNA strands, not just one, in contrast to prokaryotes, which can only have one.
    - DNA polymerase III adds dNTPs with base pairs to the 3' end OH on the backbone of a leading DNA strand, while on the lagging strand DNA polymerase I removes the RNA primers and replaces them with DNA.
    - RNA primers are used for DNA polymerase to identify where to attach to during replication. Therefore, the leading strand should only have one primer, while the lagging strand should have multiple primers due to the formation of Okazaki fragments.
    - MCM maintenance (minichromosome maintenance) prevents the re-replication of eukaryotes
    - Eukaryotic DNA is coupled to histones in packages called nucleosomes. This compacts the DNA for more efficient storage.
    - Telomeres mark the end of the chromosome in eukaryotic proteins
- Enzymes and Proteins in DNA Replication
  - Initiator Protein - binds to origin of replication and separates strands of DNA to initiate replication
  - DNA Helicase - binds to the lagging strand template at each replication fork and moves in the 5' to 3' direction, breaking the hydrogen bond and moving it along the replication fork.
  - SSBP - (Single Strand Binding Proteins) stabilize the exposed single strand of the DNA.
  - DNA Gyrase - relieves the strain ahead of the replication fork by unwinding the DNA
  - DNA Primase - synthesizes RNA primers from the DNA template strand before replication from DNA polymerase. This is required as the RNA primase serves as the starting 3'-OH group to which the nucleotides are added.
  - DNA Polymerase III - elongates the strand by adding base pairs to the leading strand.
  - DNA Polymerase I - removes RNA primers. Has 3' to 5' and 5' to 3' exonuclease activity
    - Exonuclease activity proofreads the strand by replacing mismatched bases during replication with the correct base. This is in contrast to mismatched repair, which corrects errors after replication is complete.
  - DNA Ligase - connecting nicks after the RNA primers are removed by DNA polymerase I.
  - Termination Protein - marks where replication ends.
- Differences between prokaryotic and eukaryotic DNA translation
  - In prokaryotes, transcription and translation can occur concurrently due to the absence of a nucleus. In contrast, in eukaryotes, transcription takes place in the nucleus, but translation takes place in the cytoplasm
  - Genes in prokaryotes are not divided into introns and exons, but they are in eukaryotes
  - In prokaryotes, there is only one type of RNA polymerase used for transcription, but in eukaryotes, there are different types of polymerase.
  - Primary transcripts are mRNAs with a triphosphate at the 5' end in prokaryotes, however, in eukaryotes, the primary transcripts undergo post transcriptional processing (mRNA splicing, RNA editing etc.) becoming mature mRNAs with a 5' guanine cap and a 3' poly A tail.
  - The initiator tRNA in prokaryotes carries N-formylmethionine instead of just methionine, which is the case of eukaryotic tRNA.
  - mRNAs have only one initiation site in prokaryotes, but in eukaryotes, there can be many initiation sites.
  - The small ribosomal subunit (30s) immediately binds to the ribosome binding site (AUG) whereas in eukaryotes the small ribosomal subunit binds to the 5' cap then scans the mRNA to locate the ribosomal binding site.
- Telomerase
  - Telomerase is a reverse transcriptase (RT) enzyme, similar to ones found in retroviruses in that it synthesizes new DNA strands from an RNA template. These replicate telomeres for the DNA
  - After the removeal of RNA primers, the newly synthesized DNA strand is shorter than the original DNA strand. Over many times of DNA replication, the effect becomes more pronounced. This is where telomerase comes in to fix that issue.
  - The RNA within the telomerase is complementary to the telomeres, so a part of the telomerase binds to the leading strand, then synthesizing the DNA from the RNA template (reverse transcriptase)
  - Telomeres serve to protect the free ends of DNA from fusion and recombination, like a protective cap.
  - A complex called shelterin further protects from degradation by binding onto the telomeres.
  - Lower activity of telomerase in somatic cells results in the shortening of the telomere over time.





Key People to Remember

- Arthur Kornberg - earned Nobel prize for purification and characterization of DNA polymerase and the mechanism of DNA replication
- Severo Ochoa - discovery of RNA polymerization from rNTPs (ribonucleotide tri-phosphates)
- Thomas Kornberg (son of Arthur Kornberg) - Isolated DNA polymerase III
- Carol Greider, Elizabeth Blackburn, Jack Szostak - discovered telomerase, the enzyme responsible for replication of telomeres
- Sidney Altman and Thomas Cech - Discovered the Ribozyme
- Andrew Z. Fire and Craig C. Mello - Discovery of dsRNA
- George Wells Beadle, Edward Lawrie Tatum, Joshua Lederberg - One gene, one enzyme
- Marshall Nirenberg - Determined the sequence of codons that created each amino acid through homopolymers.
- Venkatraman Ramakrishnan, Thomas A. Steitz, Ada E. Yonath - Nobel prize laurelates awarded the prize through 3D depiction of the structure of the ribosome.
- Kary B. Mullis and Michael Smith - Developed the method of DNA replication known as PCR



Lecture 8 Material
The Ames Test, Repairing DS Breaks, and CRISPR

- The Ames test uses principles of genetic screens to identify mutagens
  - Process:
    - Bacterial his- strains are mixed with liver enzymes (which have the ability to convert compounds into potential mutagenic compounds) Liver enzymes puts the mutagens in a more realistic environment, metabolizing the mutagen could potentially break down the substance into something that is mutagenic.
    - Without his- strains, the bacterial colonies are unable to produce leucine to grow in a medium that lacks histidine. We can exploit this in order to test for mutagens.
    - Some of the bacterial strains can also be mixed with the suspect chemical to test for mutagenic activity.
    - The bacteria are then plated on a medium that lacks histidine
    - Conclusion: Any chemical that significantly increases the number of colonies appearing on the treatment plate is also mutagenic and therefore probably also carcinogenic.
  - If the Ames test indicates a reversion mutation (see below), then the chemical should be mutagenic.
- Genetic Analysis of Bacteria:
  - Prototrophic - wild type
  - Auxotrophic - mutant type - unable to synthesize a particular organic compound required for its growth
  - Minimum medium - only required for prototrophic bacteria only his+ will survive.
  - Complete medium (control) - contains all substances required by all bacteria, including auxotrophic bacteria - both his+ and his- will survive
- Mutations: Forward and Reverse
  - Forward mutation - changes the wild type allele to a different allele (what one would typically think of as a mutation)
  - Reverse mutation (reversion) - changes a mutant allele back to rare (much rarer)
- DNA Repair
  - Repairing Systems:
    - Mismatch - Repairs replication errors, including mispaired bases and strand slippage
    - Direct - Repairs pyrimidine dimers; other specific types of alterations
    - Base excision - Repairs abnormal bases, modified bases, and pyrimidine dimers
    - Nucleotide excision - Repairs DNA damage which distorts the double helix, including abnormal bases, modified bases, and pyrimidine dimers
    - Homologous recombination - Repairs double stranded breaks
    - Nonhomologous end joining - Also repairs double strand breaks
  - Unrepaired double-strand breaks can lead to deletions and chromosome rearrangement (see to the right)
    - These breaks can be repaired by:
      - Homology Directed Repairs (HDR)
      - Nonhomologous End Joining (NHEJ)
  - Homology Directed Repairs
    - Involves the "invasion" of the unbroken homologous chromosome by the broken ends of the other broken homologous chromosome
    - Not as prone to errors, but more specific modification.
  - Nonhomologous End Joining
    - Does not involve a homologous chromosome, as both strands are broken
    - Often results in INDELs
    - Prone to errors (risky)
- CRISPR
  - Clustered, Regularly Interspaced, Short Palindromic Repeat
  - Based on adaptive immune responses used by the bacteria
  - Protection from invading DNA (virus or plasmid)
  - Sequences from invading DNA are incorporated between repeats in bacterial genomes
  - Transcripts from these arrays processed into CRISPR RNAs which includes the "variable sequence" (the invading sequence) which is also known as the protospacer
  - The protospacer base pairs with complementary DNA bringing the complex along
  - The Cas9 nuclease and guide RNAs are used to generate cleavage at a specific position
    - crRNA and tracrRNA form a complex with Cas9.
    - crRNA (CRISPR RNA) - an example of a guide RNA, includes a protospacer sequence designed to base pair with targets
    - PAM - another example of a guide RNA (proto-spacer adjacent motifs)
    - If the sequences are adjacent to a PAM, Cas9 cleaves the DNA.
  - The uses of CRISPR
    - Widely used in basic research to generate mutations in particular genes
    - Promise of therapeutics (moral question?)
    - More accurate than restriction enzymes because guide RNAs are longer than restriction enzymes, which result in more accuracy in what sequence of genes to target
  - Risks

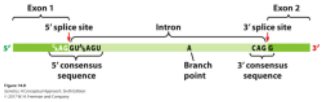
- Potential off-target effects
  - Mutations created at sites other than the intended site
  - Caused by similarity in sequence to chosen guide RNAs
  - Almost impossible to not have partial matches someplace within the genome
  - Polymorphisms (variations) mean that we cannot exactly predict sequences 100% of the time in any individual based on prior knowledge of genome sequence

Gene Structure, Transcription, Splicing, and Translation

- Classes of RNA
  - Ribosomal RNA (rRNA)
  - Messenger RNA (mRNA)
  - Transfer RNA (tRNA)
  - Minor Classes of RNA:
    - Small Nuclear RNAs (snRNA)
    - Small Nucleolar RNAs (snoRNA)
    - Small cytoplasmic RNAs (scRNA)

Lecture 9 Material
RNA Splicing and Ribozymes

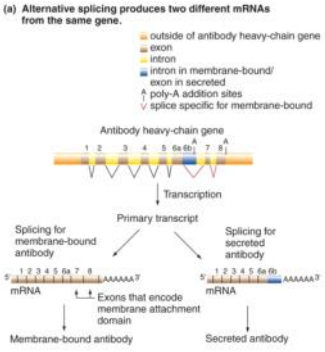
- Pre mRNA processing
  - The addition of the 5' cap:
    - A nucleotide with 7 - methylguanine; 5'-5' bond is attached to the 5' end of the RNA.
    - Facilitates the binding of the ribosome to the mRNA, increases mRNA
  - The addition of the poly(A) tail:
    - 50 - 250 adenine nucleotides are added to the 3' end of the mRNA.
    - Increases the stability of the mRNA, facilitates bonding of mRNA
- RNA splicing
  - Consensus sequences:
    - Consensus sequences tell the spliceosome where to cut the mRNA.
    - 5' consensus sequence:
      GU(A/G)AGU (5' splice site)
    - 3' consensus sequence:
      CAGG
    - Branch point: the adenine upstream of the 3' splicing site:
      - Does not have to be A, but has a strong preference for it.

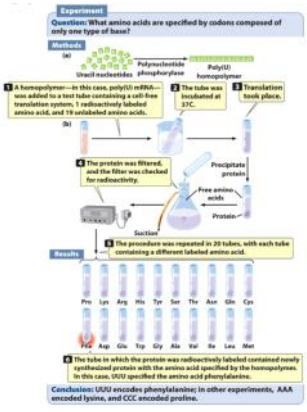


  - Spliceosome: five RNA molecules + 300 proteins
    - Splicing removes introns from a primary transcript
    - The gene is first transcripted from the template DNA strand, then the primary transcript is cut by RNA splicing, leaving justthe exons in the mRNA strand.
    - 5 snRNP proteins form the basis of the spliceosome
    - The spliceosome is responsible for holding components together during splicing reactions and mRNA splicing reactions
  - In the splicing mechanism, the 5' phosphodiester bond is broken and attached to the 2' -OH group on the branch point, resulting in the lariat. Since a bond is being formed and broken at the same time, there is no net loss or gain of energy in this transesterification process.
  - Alternate Splicing
    - Many different types of spliceosomes that can splice mRNA in many different ways
    - Allows one mRNA to encode multiple different polypeptides with different activities (can produce two different mRNAs from thesame gene.)
    - This increases the number and the diversity of eukaryotic proteins
    - Involved in cell type specificity
  - Two sequential cuts remove the intron, resulting in a loop, also known as a "lariat", an intermediate. The intron is then deg raded.
- snRNAs key to pre-mRNA splicing
  - snRNPs = proteins complexed with snRNA, responsible for recognition of 5' and 3' splice site and lariat points.
  - snRNAs that mediate pre-mRNA splicing may be related to Group II introns (may have primitive origins in Group II introns)
  - Conclusion: mRNA splicing must have arisen from ancestral self-splicing
- Self-splicing
  - Mediated by RNA alone
  - Observation of pre rRNA indicated the presence of introns
    - It was found through experimentation that the pre rRNA spliced correctly without any protein added to the reaction.
  - The discovery of this catalytic RNA is attributed to Thomas Cech. The RNA with enzymatic properties is known as a ribozyme.
- Ribozymes
  - Ribozymes are catalysts that are designed to cleave RNA of interest.
  - Pair with the target RNA
- Other types of RNA with unique activities
  - piRNA - (piwi interacting RNA) presses the expression of transposable elements
  - siRNA - (small interfering RNAs) - prevent the production of specific proteins based on their nucleotide sequence of the corresponding mRNA in a process known as RNA interference (RNAi)
  - miRNA - (micro RNA) mediates RNA interference
    - Do not encode proteins
    - If miRNA finds a **perfectly complementary** strand in cellular mRNA, the miRISC (RNA induced silencing complex) binds to the mRNA and cleaves the bound mRNA
    - If miRNA finds an **imperfect match** in cellular mRNA, the miRISC binds to the mRNA, but is unable to cleave it. However, the attachment of the miRISC prevents the progression of ribosomes down the strand, resulting in the decreased translation of mRNA.
      - These cleaved halves of RNA are no longer protected by cellular RNases and the mRNA is degraded.
    - Created (transcribed by) RNA pol I, but around 25% are generated from introns that have not degraded.
    - Highly conserved throughout evolution
    - Transcription process of miRNA generated first from "stem-loop" structures:
      1.) Stem loops on primary transcripts triggers processing
      2.) Drosha RNase binds to the stem loop, cleaving the base of the stem loop, generating this pre miRNA
      3.) The pre miRNA is transported out of the nuclear export
      4.) Dicer RNase binds to the stem loop, trimming ends to produce a 12-24 nucleotide miRNA duplex.
      5.) Dicer is incorporated into the RISC (RNA induced silencing complex)
      6.) One strand is degraded to yield a single strand of RNA loaded into RISC
      7.) miRNA is now active and ready to degrade other cellular RNAs
  - asRNA - (antisense RNA) - used to "knockdown" gene expression
  - dsRNA - (double strand RNA) - an RNAi mechanism for gene-specific silencing in organisms.
    - First discovered and isolated within nematodes
    - In subsequent tests, dsRNA was fed to the nematodes and produced expected phenotypes.
      - Systemic effects required the spreading of dsRNA via an RNA transporter
    - RNA dependent RNA polymerase amplifies the signal of dsRNA
    - Transporter proteins SID-1 and SID-2 allow dsRNA to enter new cells
    - 10 - 100 times more effective in producing silenced phenotype than antisense RNA
    - Dicer and RISC recognize dsRNA and will target cellular RNAs along with it.
    - Cells may respond to a viral attack through destruction of viral RNA
    - Enzymes segment dsRNA then chop up matching viral mRNA.
    - Process of dsRNA destroying mRNA:
      1.) dsRNA binds to Dicer
      2.) Dicer cleaves dsRNA into smaller fragments
      3.) One RNA strand from the original dsRNA - Dicer complex is loaded into a RISC.
      4.) The RISC links the compound to the mRNA strand through base pairing.
      5.) mRNA is cleaved and destroyed.

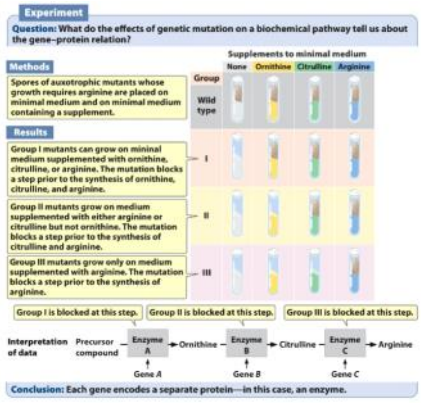
Lecture 10 Materials
RNA Activities, reviewed, and Translation.

- Eukaryotic pre-mRNA processing
  - 5' cap
    - Contains a nucleotide with 7-methylguanine; a 5'-5' bond is attached to the 5' end of the RNA
  - Poly(A) Tail
    - 50-250 adenine nucleotide structure located at the 3' end of the mRNA.
  - Splicing
    - Generates multiple RNAs and proteins from a single coding region
    - Utilizes RNA-protein complexes.
- One Gene, One Enzyme
  - Discovered jointly by George Wells Beadle, Edward Lawrie Tatum, Joshua Lederburg.
  - Genes affecting one step in the pathway was mapped to the same chromosomal locations in the experiment shown on the right
    - The wild type autotrophic mutant was able to grow on the ornithine, citrulline, and arginine, however, group I, group II andgroup III was unable to grow in the ornithine, citrulline + ornithine.
- Biochemical methods
  - Nitrocellulose filter binding assay - used for immobilizing proteins. Most proteins have a net positive charge, and the nitrocellulose filter paper has a net negative charge. DNA is negatively charged due to the phosphate backbone, however, if any protein is bound to the protein , it will be isolated
  - Trichloroacetic Acid (TCA) precipitation of proteins is used to concentrate protein samples or remove contaminants.
  - Nirenberg & Ochoa - Tested amino acids that were specified by codons composed of only one type of base.
    - Polynucleotide phosphorylase converts individual nucleotides into a homopolymer
    - Homopolymers are molecules of polypeptides which only contain one monomer which repeats many times
    - Experiment is outlined in the diagram to the right.
- Formation of a protein
  - The ribosome transcribes the mRNA from the 5' to the 3' direction
  - The polypeptide is then formed from the matching of the anti-codon to the codon in the mRNA strands.
  - This polypeptide chain formed is known as the primary structure of a protein, just the combination of the amino acids
  - The secondary structure of the protein can be either an alpha helix or a beta pleated sheet.
  - The tertiary structure of the protein is the folding of the secondary structure even further into a distinct shape.
  - (Optional) Quaternary structure includes the binding of two polypeptide chains together.
- Amino acyl-tRNA synthetases
  - 20 different amino acyl-tRNA synthetases specify the 20 different amino acids.
  - Amino acyl-tRNAs synthetases provided specificity for the different codons that code for the amino acids.
- Pairing an amino acid with tRNAs
  - First, the amino acid reacts with the ATP, dephosphorylating it. This creates aminocyl-AMP.
  - Aminocyl-AMP then connects to the tRNA through the AMP connection, which releases AMP.
- Eukaryotic translation
  - Similar to prokaryotic, but contains more proteins and differences.
  - 5' cap mediates the initiation complex formation
    - Cap binding complex is involved in translation initiation and export of mRNA from the nucleus into the cytoplasm.
    - Initiation complex scans the 5' UTR for the start codon
    - The Kozak sequence facilitates the identification of the start codon.
      - The first AUG after the Kozak sequence is the site of the start codon.
    - Poly-A Tail - protein-protein interactions facilitate the binding of ribosomes to the mRNA

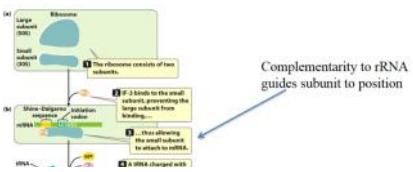




Nirenberg and Ochoa's experiment



Initiation:



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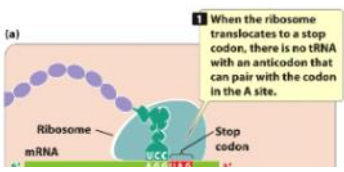

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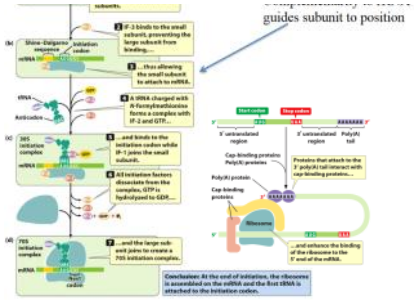
    - Poly A Tail - protein-protein interactions facilitates the binding of ribosomes to the mRNA.
- Initiation process
  - In prokaryotes, rRNA consists of two subunits - the large subunit (50S) and small subunit (30S)
  - Process of initiation:
    1.) IF-3 protein (initiation factor 3) binds to the small subunit (30S), preventing the large rRNA subunit from binding to the smallsubunit, allowing for the rRNA to attach to the mRNA at the Shine-Dalgarno sequence.
      - UAAGGAGGU is the sequence
      - The initiation codon following this sequence in the 5' to 3' direction is where transcription of the mRNA begins.

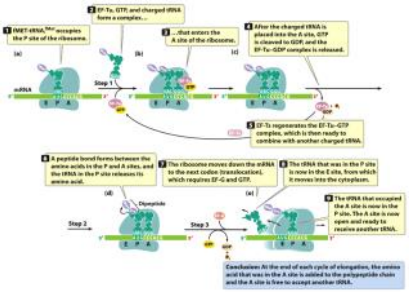
    2.) A tRNA charged with N-formylmethionine (fMet) forms a complex with IF-2 and GTP
      - N-formylmethionine is like the regular start amino acid (Met) but is specifically used for the initiation of protein synthesis from bacterial genes

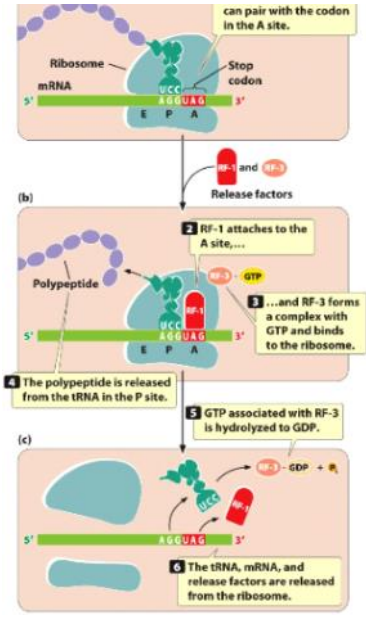
    3.) The tRNA-IF2-GTP complex binds its anticodons onto the start sequence on the mRNA
    4.) The IF-1 protein binds to the small subunit rRNA. At this point, the rRNA - tRNA complex is known as the "30S" initiation complex
    5.) All initiation factors dissociate from the complex (except fMet) and GTP is hydrolyzed for its energy to form GDP.
    6.) The large subunit (50S) ribosome attaches to create a 70S initiation complex. The rRNA is ready to start reading the mRNA
  - In eukaryotes, instead of the Shine-Dalgarno sequence, the Kozak sequence is used to identify the start codon.
  - Eukaryotes also have special cap-binding proteins that facilitate the initiation of reading.
    - The poly A tail bends toward the cap-binding proteins, facilitating further the binding of the rRNA to the 5' end of the mRNA.
- Elongation of mRNA
  - Three main sites on the ribosomes
    - Aminoacyl site A, Peptidyl site P, exit site E
    - Order:
      5' end ---- E----P----A----3' end
  - Process of elongation:
    1.) fMet-tRNA complex occupies peptidyl (P site) in ribosome
    2.) The EF-Tu (elongation factor thermo unstable) G-protein, GTP, and a new charged tRNA forms a complex. This tRNA then moves to the A site in the ribosome.
    3.) GTP is cleaved to GDP, and the EF-Tu-GDP complex is released from the tRNA. EF-Ts then regenerates the EF-Tu-GTP complex from the EF-Tu-GDP, starting the process again to attach to other tRNA.
    4.) A peptide bond forms between the two amino acids in the P and A positions in the ribosome, and the tRNA in the P site releases its amino acid.
    5.) EF-G (Elongation Factor G or translocase) and GTP moves the tRNA in the P position toward the 5' end to the E position, from which it moves into the cytoplasm of the cell. The tRNA in the A position now moves to the P position, and the process can be repeated
- Translation termination:
  - There are no tRNAs with the complementary anticodons for UAA, UAG, and UGA. This leads to the stop of translation.
  - Process of termination:
    1.) When the ribosome translocates to a stop codon, there is no matching tRNA that can pair with the codon at the A site.
    2.) RF-1 attaches to the A site in the ribosome, corresponding to the stop codon on the mRNA.
      - Note - different stop codons are recognized by different release factors.

    3.) RF-3 forms a complex with GTP and binds to the large subunit of the 70S ribosome.
    4.) The polypeptide chain is released.
    5.) GTP-RF-3 is hydrolyzed to form GDP-RF-3, releasing the tRNA, RF-1, and the ribosome from the mRNA.
- Evidence for these processes:
  - In vitro reconstitution and genetics
  - Negative controls include
    - Individual tubes that leave out the protein, mRNA, tRNA, or at different temperatures to test if the reaction took place dueto experimental conditions rather than confounding variables
- Factors that regulate translation
  - Proofreading - ribosome "checks" for mRNA and tRNA mismatches, which can cause premature termination in bacteria.
  - Polyribosome (polysome) - a complex of mRNA and several ribosomes that act to translate mRNA instructions to polypeptides.
    - Same mRNA occupied by multiple ribosomes, resulting in the co-translation of mRNA.
  - mRNA surveillance
    - Detect and deal with errors in mRNA
    - Nonsense-mediated mRNA decay (NMD): elimination of mRNA containing premature termination codons.
    - Absence of stop codons may result in stalled ribosomes
      - Stalled ribosomes are removed in bacteria
      - Eukaryotes have nonstop mRNA decay
      - NO-GO decay - removes stalled ribosomes from stalling due to their secondary structure.



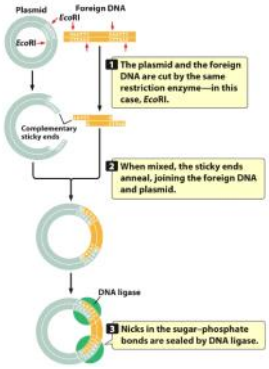
Elongation:





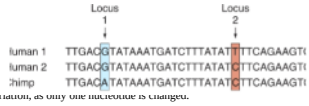
Lecture 11 Materials
Molecular Methods and Genetic Analysis

- Recombinant DNA technology
  - Restriction enzymes
  - Plasmid vector
  - PCR and cloning by PCR
- DNA sequencing
  - Sanger sequencing
  - Next generation sequencing
- Restriction enzymes
  - Also known as endonucleases, recognizes and cuts DNA at specific nucleotide sequences. Examples include:
    - Bam HI
    - Hind III
    - Eco R1
  - Highly specific, highly efficient
  - Allows us to put specific DNA fragments together in combinations which researchers choose
  - Different restriction enzymes cut at different spots, resulting in either blunt or sticky ends.
    - Sticky ends - staggered cuts in DNA that can be paired together using DNA ligase
    - Blunt ends - non-staggered cuts that cut straight across both strands of DNA.
  - Whenever the restriction enzyme encounters the specific sequence, it will cut at the sequence.
- Plasmid vector
  - Plasmids - circular DNA molecules forming from bacteria
  - Foreign DNA is inserted into the plasmid using restriction enzymes.
  - Polylinkers - (Multiple cloning sites) synthetic DNA that contains restriction sites
  - Expression vectors - include specific DNA sequences to allow for the expression of certain genes in different organisms.
  - Process of insertion of DNA fragments into a vector:
    1.) Digest plasmid with restriction enzyme
    2.) Remove 5' P ends (phosphatase)
    3.) Digested foreign DNA with the same restriction enzyme
    4.) Purify each DNA piece
    5.) Join the pieces together with DNA ligase
    6.) Spread colonies on plate
- Polymerase Chain Reaction (PCR)
  - Process of PCR:
    1.) DNA is heated to 90 to 100 degrees C to separate the original DNA strands
    2.) DNA is cooled to 30 to 65 degrees C to allow the short single stranded primers to anneal to their complementary sequences
    3.) The primer and DNA polymerase are added to synthesize new strands from the old DNA.
  - Each cycle of PCR doubles the amount of DNA
  - The Taq polymerases added to the PCR are isolated from bacteria which can withstand very high heat. Therefore, the polymerase is not denatured in the high temperatures.
  - Oligonucleotides - used in place of primers that can be used to replicate particular target DNA sequences.
- Sanger Sequencing
  - Involves the incorporation of a dideoxynucleotide (ddNTP) to terminate DNA synthesis. This creates a series of single strande d DNA fragments.
  - The DNA fragments are a nested array - differing in length from the preceding and succeeding fragment by one nucleotide
  - In gel electrophoresis, the sequence represented on the gel is the complementary strand of the DNA.
- Next Generation Sequencing
  - High throughput or massively parallel sequencing - large amounts of DNA added and sequenced by sequencing libraries or adding adapters of known sequences.
- Antibiotics
  - Antibiotics most often inhibits prokaryotic translation
  - Antibiotics are used to study translation and as tools in recombinant DNA technology.
  - Puromycin is an example of an antibiotic that is both an antibiotic and an anticancer drug



Lecture 12 Materials
Chromosomal Variation

- Short Tandem Repeats or Simple Sequence Repeats (STRs and SSRs)
  - There are various numbers of STRs/SSRs in each human in specific loci
- Polymorphisms
  - Most polymorphisms are in non-coding regions of the DNA
  - Genomic regions are aligned based on high degrees of similarity
  - SNPs (single nucleotide polymorphisms) are the most common type of variation, as only one nucleotide is changed.
  - CNVs are the rarest form caused by unequal crossing over.



- Aneuploidy is an increase or a decrease in the number of individual chromosomes
  - Nullisomy - loss of both pairs of a homologous chromosome
  - Monosomy - loss of a single chromosome
  - Trisomy - gain of a single chromosome
  - Tetrasomy - gain of two homologous chromosomes
  - Polyploidy - the presence of more than two sets of chromosomes
    - Common in plants
    - Increase in cell size
    - Larger plant attribute
    - Evolution - may give rise to new species
    - Autopolyploidy - from a single species (ex. sunflower)
    - Allopolyploidy - from more than one species (ex. bread wheat)