

Prokaryotic Transcription--Initiation

Prokaryotic Transcription--Elongation and 2 Types of Termination

DNA Replication (Including Replicons, Fidelity, and Initiation, Elongation and Termination Steps)

Reverse Transcription and Telomeres

- Elongation and Translation occur simultaneously in prokaryotes, with elongation occurring at a rate of ~40 NT/second.
- Termination allows regulation of various operons.
 - * Rho-independent termination occurs when the nascent RNA strand forms a hairpin structure, causing polymerase to pause. A nascent multi-U sequence in the mRNA then forms an unstable duplex with the DNA, and falls off all at once, at which time the polymerase is released.
 - * Rho-dependent: rho is an RNA-dependent ATPase and helicase. The ribosome is normally right behind the RNA polymerase on the nascent strand, leaving no room for rho to attach. When the ribosome hits a certain sequence on the mRNA it pauses, creating a loop of mRNA to which rho can bind, chase down polymerase, and smack it off of the DNA.

- Most of the bacterial genome is transcribed (coding). The transcription unit in prokaryotes is the operon, which consists of a promoter, the code, and a terminator.
- RNA polymerase binds to the promoter region, which includes several sequences recognized by the sigma subunit (Pribnow Box and -35 sequence). Other sigma subunits bind to other promoter sites.
 - * Prokaryotes have only one type of polymerase. The active core enzyme (polymerase) together with a specific sigma factor = the holoenzyme.
- The Sigma subunit binds by sliding along the DNA, forming a closed complex on the promoter. When it hits the promoter, the DNA "melts", exposing it.
- A ternary complex of the DNA, polymerase and first RNAs form.
- Sigma is released and other elongation factors join in.

- Retroviruses, which are like transposons that have gained the ability to leave the cell, encode a reverse transcriptase that copies RNA into 2-stranded DNA.
- Telomerase is a special form of reverse transcriptase.
 - * Telomeres appear at the end of Euk chromosomes and are short sequences of NT's repeated ~2000 times.
 - * Because Okazaki fragments require an RNA primer, the final one begins before the end of the linear template, and some of the telomere is lost.
 - * Telomerase uses a bound RNA template to form new DNA telomere repeats, which are then matched on the opposite side by DNA polymerase.

- A unit of DNA replicated in one go is called a replicon. In Proks it's a circle, in Euks there are multiple replicons per genome.
- Fidelity in replication comes from b.p. specificity, a 3'-5' exonuclease, and DNA repair mechanisms.
- Initiation: Protein machinery is assembled at the replicon origin.
 - * Helicase unwinds, topoisomerase removes supercoils, binding proteins maintain the DNA in its unwound state, and primase creates RNA primers for DNA polymerase.
 - * In Euks, the Origin Recognition Complex binds the origin and opens the fork.
- Elongation: Polymerase sees the 3' -OH group on the primer and begins synthesizing in the 5' to 3' direction.
 - * The lagging strand is also synthesized in the 5' to 3' direction, "doubling back" to remove the RNA primers in front of each Okazaki fragment and ligate together individual DNA fragments.
- Termination: Bacteria have 1 point of origin and 1 termination region. When the forks going opposite

Eukaryotic Transcription II

Cis-Acting Elements Regulating the Average
Transcription Unit

5 Steps of Eukaryotic Transcription & Post-
Transcriptional Processing

Eukaryotic Transcription I

- Promoter
- Enhancers
- LCR
- Splice donor and acceptor sites
- Cleavage/Poly-adenylation site

- Possible models for transcriptional activation:
- Co-activators and GTFs lead to efficient assembly of initiation complexes.
- Pre-initiation complex's conformational change leads to more binding of GTFs.
- Covalent modification of proteins in the basal transcription machinery.
- Chromatin surrounding promoter may be modified to increase accessibility.
- An LCR is sometimes required for activation of genes at a distance, and is bound by different activators at different points. It acts as a global regulator.

- As in prokaryotes, activators and repressors stimulate or block transcription.
- Unlike in prokaryotes, there are many more proteins involved, and chromatin generally represses transcription.
- The promoter includes all sequences involved in initiation, both near and far.
- TATA box is at the initiation site and is part of the core promoter.
- Enhancers can be kb away from the core promoter, in any direction. They may raise transcription in certain genes, or at certain times.
- General Transcription Factors are complexes that bind TATA and polymerase into a holoenzyme.
 - * The Tata Binding Protein provides the promoter recognition of sigma. GTF (including TBP) recruit polymerase.
 - * The holoenzyme binds with TBP (which is bound to TATA box, and recruits the holoenzyme), and begins to transcribe the mRNA, while TBP stays on the TATA box.
- Gene-specific transcription factors include DNA-binding

- 1) Initiation of RNA synthesis by RNA polymerase II in response to enhancers and regulators and repressors that can be near or far to the start codon.
- 2) Addition of 5'-5' linked cap and methylation 5' from the TATA box and start codon--stabilizes mRNA in the cytosol & helps bind ribosome.
- 3) Splicing of pre-mRNA to remove introns
- 4) Poly-A addition to 3' end of mRNA--involved in translation.
- 5) Transportation to cytoplasm.

Translation: Elongation & Termination

General Differences in Eukaryotic and Prokaryotic Translation

Eukaryotic mRNA vs. Prokaryotic mRNA

Translation Initiation in Prokaryotes and Eukaryotes

- Euks have bigger ribosomes
- Euks use the capped mRNA and the loop it helps make as recognition features for binding
- Euks have a lot more Initiation Factors
- Euks have a single Release Factor that recognizes all three stop codons.

- A site loading: Selection and proofreading of A.A. Can be disrupted by drugs like aminoglycosides which increase errors in tRNA miscoding.
- P site joining: A.A. amine attacks carbonyl, tRNA is released through E site, amide/peptide bond is formed in dehydration synthesis. Some drugs block peptide bond formation directly, others block tRNA from leaving through exit channel.
- mRNA-tRNA complex "bumps over" to free up A site.
- Termination: Stop codon is recognized by a Release Factor, which stimulates hydrolysis of the growing peptide chain. Other release factors split up the ribosomal units and they travel on their merry way.

-Prokaryotes: SD interaction is engaged, 3 Initiation Factors help bring the tRNA(met) and ribosomal small subunit over to the mRNA.

*tRNA(met) is placed at the P site and GTPase brings over the large subunit.

* Multiple ribosomes can bind to various SD sites on the mRNA, translating the multiple proteins on one polycystronic mRNA at once, or one protein multiple times.

-Eukaryotes: Complex of proteins binds the cap and polyA tail to form a loop.

* IF2 binds and helps locate AUG by scanning the loop 5'-3' from the cap, tRNA(met) is deposited, and large subunit joins.

- Euk has a cap, encodes a single protein, and has a poly-A end that's not coded in the DNA.
- Proks include Shine-Delgarno sequences (that allow mRNA to be tethered for translation to begin) and start codons in front of each of several proteins coded in a single polycystronic mRNA.
- Comparison picture.

Peptidoglycan

Gram+ vs. Gram- Bacterial Envelope

Wobble Pairing

General Features Distinguishing Prokaryotes from Eukaryotes

- Look at picture from notes.
- Gram- bacteria have two membranes, the second of which contains LPS. LPS contains Lipid-A (the cell's endotoxin and a permeability barrier to large or hydrophobic molecules), a core, and a species-specific repeating polysaccharide sticking out into the medium (O-antigen).
- Gram+ bacteria have super-thick peptidoglycan wall, which prevents gram stain (purple) from escaping. They have lipoteichoic acids that are involved in antigenic identification.
- Gram+ bacteria have teichoic acids extending out from both the membrane and the peptidoglycan, which is immunogenic.

- Chains of alternating sugars connected by peptide bridges.
- In Gram+ bacteria, an alanine is cleaved off the five-aa chain and then the fourth alanine is connected via 5 glycines to the third aa (lysine) on the adjacent sugar chain.
- In Gram- bacteria, an alanine is cleaved and then the remaining alanine is connected to a DAP in the adjacent chain via a single aa bond.

- No nuclear membrane
- Usually a single circular chromosome
- Genes uninterrupted by introns
- Ribosome 70S, not 80S
- Cell walls contain muramic acid
- No mitochondria or membrane-bound vesicles
- No cytoskeleton or endocytotic vesicles
- Movement by flagellar rotation
- Cell envelope of 1 or 2 membranes plus peptidoglycan wall
- Glycocalyx or capsule: slimy layer of polysaccharides

- Involved in the "degeneracy of the code".
- Allows 64 codons to be read by many fewer tRNAs.
- The third position (3' end of mRNA codon, 5' end of tRNA) doesn't really matter too much.

Function of the Capsule

Levels of Gene Regulation

Penicillin, Penicillin Resistance, and Penicillin-Derivatives

Vancomycin & Vancomycin Resistance

- Transcription initiation: Positive or negative regulation by trans-acting factors on the promoter.
- Transcription elongation/termination: Secondary structures that influence RNA polymerase.
- Translation initiation: Structures that regulate access to the Shine Delgarno sequence (in proks).
- Translation elongation: Ribosome pausing, attenuation.

- Usually polysaccharide, can contain protein or glycoprotein.
- Species-specific!
- Functional roles:
 - * Attachment: adhere to things and form biofilm, which can provide antibiotic resistance.
 - * Protection: from phagocytotic engulfment.
 - * Resistance: to drying.
 - * Reservoir: for some nutrients.

- Vancomycin, like pen., targets cross-linking of the peptidoglycan side chains. Unlike pen. it binds the terminal D-ala-D-ala directly and sterically prevents its cleavage by the transpeptidase.
- Gram- bacteria are innately resistant to vancomycin b/c it's too large to pass through the outer membrane.
- Resistance occurs from a multigene operon, the net effect of which is to replace the terminal D-ala with D-lac. Crosslinking can still occur, but vancomycin no longer binds.

- Transpeptidase catalyzes release of 1 ala, then attack by the amine from DAP or the glycine bridge on the remaining Ala carboxy terminal.
- Penicillin's B-Lactam rings mimic the D-ala-D-ala and inhibit transpeptidase by permanently binding to the enzyme.
- Because the cell wall cannot be reinforced with the peptidase bridges, the cell lyses.
- Penicillin resistance is usually a B-lactamase that cleaves penicillin. Gram- bacteria are innately resistant to penicillin because of LPS.
- We can fight resistance by changing the side chains to form, say, methicillin, which also works against Gram-bacteria because it passes through pores in the outer membrane.

Regulation in Prokaryotes by Specialized Sigma Factors

Attenuation Mediation of Transcription (in prokaryotes)

Operons and Regulons in Prokaryotes

The Lac Operon as an Example of Regulation

- Only possible b/c translation occurs simultaneously.
- trp operon example: Consists of a leader region and structural genes for synthesizing trp.
 - * In a high-trp environment, the leader region (consisting of two codons for trp) is transcribed, and the terminator region of mRNA following forms a hairpin loop for rho-independent termination of transcription.
 - * In a low-trp environment, the ribosome stalls at the leader region (b/c it has no trp), newly synthesized mRNA forms a different "antiterminator" loop, the RNAPolymerase is able to continue onto the body of the trp operon (which codes for the production of trp), and the ribosome is free to follow along and continue translating.

- Sigma factor initiates transcription by joining RNA polymerase and binding to the Pribnox box and -35 sequence.
- Sigma factors in the RNA polymerase holoenzyme can be replaced sequentially in response to different biological stimuli.
- For example: when sporulation genes need to be expressed, they require the prior translation/expression of specialized sigma factors.
- Different sigma factors recognize different promoter sequences.

- An operator/promoter allows RNAPolymerase in the absence of a repressor protein.
- The lac operon includes a Lacl regulator and 3 structural genes.
- When Lacl is expressed, there is no RNAPolymerase binding to the promoter b/c Lacl binds the promoter and blocks RNAPol.
- In the presence of lactose (the inducer), lacl no longer binds to the operator/promoter, and expression of the operon occurs (which results in synthesis of lactose).
- CAP, on the other hand, is an activator. When glucose IS in the medium, cAMP levels are low. When there is no glucose around, cAMP levels are high, which leads to activation of the lac operon and the production of B-galactosidase (to use lactose).

- Operons include genes for multiple enzymes in a single pathway and are clustered together on the chromosome and transcribed and expressed together.
- Promoter is a DNA sequence 5' from the operon.
- There is a selective advantage of co-regulation as it allows multiple genes to be transcribed without requiring as many regulatory elements.
- A regulon is a collection of genes scattered around the chromosome that are still coordinated by a single repressor or activator, and have the same promoter.

How to Isolate Mutants

Mutation Types and Causes

Translation Release Factor Example of Self-Regulation
in Translation

Quorum Sensing

- Missense: Replaced amino acid.
- Nonsense: Stop codon created, either by a point mutation or a frame shift.
- Frame shift: Leads to missense and nonsense codons.
- Silent: Codon changed but amino acid sequence remains the same.
- Many point mutations are conservative, either because they result in the same aa being coded, or because the new amino acid is chemically similar to the old one (conservative nature of the code).

- The easiest way is direct selection: pour on antibiotics, and only those bacteria that mutate a resistant gene will survive.
- You can use successive cycles of enrichment to select for auxotrophs, as penicillin will not kill any bacteria that are not dividing (b/c they can't synthesize whatever AA is absent from the medium).
- Replica plating: Grow colonies with a supplementary compound, use velvet to replica plate them onto plates w/o that compound, and see which colonies fail to grow.

- Prokaryotic cell-cell communication that allows behavior (expression) modification as a function of cell density.
- Underlies biofilm formation, virulence, etc.
- Staph A: At low concentrations, bacteria express proteins for adherence and concentration. At high levels, they express proteins for toxicity and proteases.
- Utilizes a 2-component signaling pathway with a sensor histidine kinase autophosphorylating and then passing messages along to a DNA-binding/transcriptional activator domain.

- If there's sufficient RF floating around, the stop codon in the middle of the gene encoding RF2 ends translation, producing a nonsense fragment.
- If levels of RF2 are low, the ribosome "slips" over the stop codon, and continues on to produce the finished protein.

DNA Repair Strategies

Recombination: General and Specific (Generally Speaking)

Phenotypic Return to Wild-Type: Reversion and Suppression

Chemical and Radioactive Mutagens

- General recombination takes place when 2 DNA duplexes with a homologous region form a heteroduplex joint, which can slide along, and is later recut. Depending on which direction the two are sliced, we may or may not have recombination outside of the homologous region.
- See picture in notes or on card.
- Site-specific recombination occurs w/ mobile genetic elements, and only genes located near the specific integration site will be moved.

- Direct repair: Thymine dimers are fixed with white light, alkyltransferase de-alkylates guanine.
- Base excision repair: Chemically modified base is cut off.
- Nucleotide excision repair: Entire mispaired nucleotide is removed. If this occurs during DNA replication, the template strand is always preferred for mismatch repair because it's already methylated.
- Homologous recombination with another duplex (including the other daughter strand) can somehow correct errors.

- 4 classes of chemical mutagens:
 - 1) Base analogues: incorporated during replication and ambiguously bind, leading to missense mutations.
 - 2) Deaminating agents: C-> U or A-> random thing that binds C.
 - 3) Alkylating agents: Methyl- or ethylation leads to mispairing and lost bases.
 - 4) Intercalating agents: Stabilize frameshifted mispairing
- Radiation causes breaks which can be mis-repaired, and also causes thymine dimers, which are bad.
- The same sort of mutagen that caused a mutation is usually the kind that might revert it (white light for UV damage).

- Reversion refers to an actual return to the W.T. gene sequence (A mutated to G mutates back to A).
- Suppressors can be intragenic or extragenic.
 - * An intragenic suppressor would be a temperature-sensitive missense mutation that is fixed by a second AA substitution that increases thermal stability, or a -1 frameshift "fixes" a +1 frameshift elsewhere.
 - * Extragenic suppression would be the mutation of a separate protein that allowed the original protein to again function. If one protein in a dimer mutates, mutate the other one to match.
- Translational suppression: Fix a nonsense mutation by altering a tRNA's anticodon to allow it to stick to some random A.A. onto the stop codon site.

Transposition

Koch/Molecular Koch Postulates

General Methods of Drug Resistance

F and R Factors

- Proof of causality for a particular microorganism or (genetically) potential virulence factor.
- Bacteria must be present in all cases, gene must be present in all virulent strains.
- Gene must be absent in avirulent strains.
- Disruption of the gene must reduce virulence.
- Re-introduction of a cloned gene must restore virulence.
- The microorganism must be able to be isolated and grown.
- Injecting the microorganism into a healthy animal must reproduce disease.

- A transposon is a DNA segment able to move to a new site in the host genome w/o any sequence homology.
- They encode the machinery for their own movement.
- After insertion, gaps are filled in, leading to target site duplication (a hallmark of transposition).
- Often many copies per cell.
- They sometimes activate or inactivate neighboring host genes when inserted.
- They include both inverted and direct repeats.
- Insertion Sequence elements are the smallest unit of transposons in bacterial cells. They don't carry resistance genes, but do carry genes to move themselves around.
- Can "hop" from chromosomes to plasmids or phage genomes.

- Conjugation involves uni-directional transfer of information.
- F-factor is a plasmid that carries genes that allow it to be transferred via conjugation (sexual reproduction). It codes for the f-pilus, which attaches to specific receptors on F- cells.
- F' occurs when some bacterial DNA is incorporated into the F-loop, and then the areas right around it are sometimes transmitted. Only the flanking regions are transferred.
- High Frequency Recombination occurs when F loop is incorporated into bacterial DNA.
 - * During conjugation, DNA transfer begins in the middle of the f-factor (at the *oriT* marker) and proceeds in one direction. Because it doesn't make it all the way around, some host genes are transferred INSTEAD of the F-factor.
 - * Recipient bacteria no longer become F+, because they don't get the whole F+ genes.
- Using independently derived HFR strains allows for

- Enzyme may alter drug structure (penicillin is cleaved, neomycin is phosphorylated, for example).
- Alteration of the drug target structure (as in vancomycin resistance and the replacement of a D-ala with D-lac).
- Alteration of membrane behavior (as when tetracycline is pumped out of the cell).
- Drug-resistant form of the enzyme.

Salmonella, Pathogenicity Islands, and Type III and IV Secretion

Lytic vs. Lysogenic Lifestyle

Toxins

Pathogenicity Islands

- Upon entering the cell, the bacteriophage makes a "choice" (essentially randomly, though a lysogenic virus will generally be predominantly in that form).
- The lytic choice consists of replication of phage DNA, expression of viral proteins, assembly of progeny, and lysis of host cell (releasing progeny).
- The lysogenic choice consists of the phage genome entering dormancy and replicating once per division, either extra- or intra-chromosomally.
- These two lifestyles can be used as a "phage test" alternative to the Ames test for mutagenicity.

* Infect bacteria with a phage, which will initially be predominantly in the lysogenic form. Expose the bacteria to a potential mutagen, which will damage the bacterial DNA and induce the SOS DNA-repair response. The SOS response, while activating DNA repair, also cleaves the viral repressor that is maintaining it in the lysogenic stage. Thus, some virus particles will enter the lytic stage, causing lysis and forming plaques on the plate. The relative mutagenicity of the compound can be measured by looking at the number of Plaque-Forming-

- Salmonella need to accomplish two things to cause typhoid: 1) Cross into the cell, and 2) Replicate w/i cell w/o being killed.
- By randomly inserting IS elements into genes you can find which genes are involved in invasion. These genes are responsible for getting through the epithelium, and are clustered at a chromosomal locus named SPI-1.
- Another set of homologous genes, SPI-2, are responsible for intracellular survival.
- SPI-1 and SPI-2 are components of a specialized secretory apparatus that delivers toxins to the host cell (type III secretion).
- Type III secretion is a hallmark of Gram- virulence, and consists of indirect delivery--proteins are secreted from the bacteria that form a pore for the toxin to be passed into the host cell.
- Type IV system is analogous to pili, and injects directly into the host cell.

- Clusters of genes encoding virulence-related functions.
- Often transposon-associated elements w/i the island.
- GC content is often different from the bacteria as a whole, suggesting it originally came from a phage or other species.
- Often encode type III or IV secretion apparatus, and may also encode the secreted effectors.

- A classic virulence factor is an exotoxin.
- A-B toxins have two parts: the toxin and the binding part.
 - * Cholera toxin B-subunit binds to a receptor on the small intestine enterocyte, leading to internalization of both A + B.
 - * the A-subunit ribosylates G-protein alpha subunit, making it always on, which causes cell issues.
- Cholera toxin is encoded in the cholera gene in a transposon, which is actually the genome of a bacteriophage that infects *V. cholera*.

Transduction and Transformation

- General Transduction: Segment of bacterial genome is erroneously packaged in a phage instead of the phage genome. Any section of the bacterial genome can be packaged and transferred to another bacteria in this way.
- Specialized Transduction: the prophage imperfectly excises itself from the bacterial chromosome, carrying some flanking DNA with it. The frequency of genes co-traveling is a measure of their proximity to the phage's insertion site, and can be used to develop a map of the bacterial genome.
- Transformation: Direct uptake of free DNA. This is inefficient and only occurs at certain parts of the cell's replicative cycle. The double-stranded DNA is cleaved at the membrane, one enters the cell, and displaces its homolog in the bacterial chromosome.