20.106J – Systems Microbiology
Lecture 7
Prof. DeLong

- Problem set 2 due today.
- For next week: we'll be doing Chapter 8 of Brock
- Exam in a week and a half

- Slide: Central Dogma
  - Replication, Transcription, Translation
  - Reverse transcription sometimes
- Slide: Flow of information
  - Right now we're just thinking about information flow within an individual species – how nucleic acid information flow occurs
  - How you maintain fidelity and copy your DNA for all your daughter cells
- What we know about DNA:
  - The four bases
  - The ring system of the sugar
    - The number of carbons
    - How you tell the difference between DNA and RNA (the lack of oxygen)
  - The nomenclature of the bases
  - Alternating linkage (3' and 5')
  - The base pairing (G-C, A-T) –This is how it replicates with such high fidelity.
  - Anti-parallel (5' → 3', 3' → 5')
  - When the structure was first determined, the pairing was inferred, but the method of replication wasn't clear
    - It could have been Semiconservative, Conservative, or Random Dispersive.
    - These different methods incorporate the mother strands into the daughter strands in different ways.
    - To determine this, they added ^{15}N labels experimentally – they could predict the densities resulting from each replication method – heavy vs. light
    - The Conclusion, of course, was that it was semiconservative.
  - The replication process is not simple, and involves a lot of enzymes.
    - Polymerase III can't just set down on a DNA strand – it needs a DNA primer. That primer is then used to extend the DNA strand.
    - This means that initially it's not just a DNA strand – it's a DNA-RNA hybrid.
    - The DNA polymerase synthesizes 5' → 3'. This means that one strand goes more easily than the other strand. The leading strand goes directly, but the lagging strand develops a lot of smaller fragments – these are called Okazaki fragments (about 1000 bases

long). DNA polymerase I (not III) is used on the lagging strand, so that it will eat through the extra primers (polymerase I would stop).
        - An RNA primer is laid down by a primase.
        - The primers have to be removed.
        - Nicks have to be removed by DNA ligase.
        - With a circular DNA, the replication happens circularly (a bubble opens up between the strands, and replication happens there).
            - This is generally what happens with prokaryotes.
            - But there are also some that have linear DNA.
- Regulatory Pathways in prokaryotes – the larger topic of today's lecture.
    - The more important means of cell regulation is transcription.

- Prokaryotic transcription
    - DNA is more stable than RNA, while RNA can be turned over and used faster than DNA.
    - The 2' hydroxy group of RNA is useful here.
    - RNA polymerase does not need a primer – it just transcribes directly.
    - Halfway through the sigma factor falls off.
    - Pribnow box and -35 region.
    - Some bases are more highly conserved than others. Also, some organisms are more GC-rich than others.
    - There are different types of sigma factors
        - Sigma 70 – kind of the vanilla flavor – used for "normal" promoters
        - Sigma 32 is used for heat-shock promoters
        - Sigma 54 is used for N limitation promoters
    - There's a modularity in how cells can turn on all kinds of different genes.
    - Strong versus weak promoters.
    - Initially RNA polymerase binds, the strand is opened up, and transcription begins.
    - Transcription termination
        - Inverted repeats – these connect to each other, stopping transcription, and they require no extra factors.
        - Row dependent terminations – require extra proteins
        - There are other sorts as well

- Differences between eukaryotic and prokaryotic transcription
    - Archaea are very different from bacteria – they do it more like eukaryotes.
    - Eukaryotes do not have classical operons
    - Eukaryotic mRNAs are usually sliced, capped, and tailed, in the nucleus
    - RNA polymerase structure/function differ
    - Initiation complexes differ
    - These differences make good targets for antibiotics
    - Translation – prokaryotes are better in terms of rapid response – transcription and translation happen next to each other – they are coupled. In eukaryotes, one is in the nucleus, the other in the cytoplasm.

- Overview of Prokaryotic Translation
  - Slide: simple structure of a prokaryotic gene
  - Maintaining high enough fidelity for translation is really remarkable
  - Shine-Dalgarno sequence
  - The genetic code has been inferred from lots of different experiments – which codons code for which amino acids
    - How this code could have occurred is really remarkable
    - Originally it was assumed to be random, in the 1960s, but this doesn't seem to be the case, because it seems like there is some order to the groupings.
    - Nobody really knows how it developed yet though.
  - Transfer RNAs
  - The early experiments that discovered how this works were really remarkable
  - The Genetic code is degenerate (meaning that multiple codons can code for the same amino acid).
    - Sometimes there is a bias (in once cell, for example, UUU might be used for phenylalanine more often than UUC is used)
    - Codon families exist.
    - Also codon pairs.
  - Staying in the correct frame is critical.
  - The code is not absolutely hard and fast:
    - Recently selenocysteine and pyrrolysine have been dubbed the 21st and 22nd amino acids
    - Previously people had thought that the Selenium in Selenocysteine was added later, but this is incorrect. It has its own codon (UGA – normally nonsense/stop codon!) and transfer RNA.
    - For pyrrolysine, it looks like a similar mechanism, although it's not yet absolutely hard and fast. (UAG codon)
  - The tRNA has to be very well matched (very specific covalent bond).
  - Aminoacyl-tRNA Synthetase recognizes both the amino acid and the tRNA.
    - It's a two-step reaction.
    - Anticodon loop and acceptor stem of the tRNA are both involved.
  - Messenger RNAs are very labile – they turn over on the order of minutes in the cells, getting eaten up by nucleases. Other RNAs are more stable.
  - Drugs that inhibit translation: there are a whole series of antibiotics that inhibit bacterial translation without touching eukaryotic translation.
  - Initiation
    - 50S
    - 30S
    - Shine-Delgarno
  - Elongation
    - Movement of ribosome along the strand.

        - After peptide bond formation, there's a new empty site, and the ribosome moves along so that the chain grows.
        - Incredible that the ribosome can do this with such high fidelity.
    - Ribozymes are catalytic RNAs