Lecture 1 - Overview of AMG (AMG text pp. 3-12)

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Flow of Genetic Information
Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) polymers consist of repeating units of deoxynucleotides and ribonucleotides, respectively. With the exception of some viruses, most all organisms on this planet store their cellular blueprints for life in double-stranded DNA molecules called chromosomes. In eukaryotic cells, chromosomes are copied during cell division, recombined and shuffled as a result of sexual reproduction, and transcribed into complementary RNA molecules through a process called gene expression.

Chemical information stored in the DNA coding sequences of a gene is transmitted to the protein synthesis machinery in the cell by mRNA “transcripts”. This relationship between the DNA, RNA and protein sequence information of a gene is sometimes referred to as the biochemical flow of genetic information.

Describe three levels of genomic regulation with regard to cellular phenotype, for example, in response to extracellular stimuli, or as a result of terminal differentiation?

Why is RNA instability a “good thing” in biological processes?

Chemical Composition of Nucleic Acids
Each deoxyribonucleotide unit in DNA contains one of the four bases; guanine (G), adenine (A), cytosine (C) or thymine (T); RNA contains uracil (U) in place of thymine.

Nature’s ability to encrypt the essence of life in long strings of DNA sequence, is not a problem of storage space, but rather how accurately the cell machinery is able to retrieve and interpret this vast amount of information. Applied molecular genetics is the exploitation of this knowledge to investigate and utilize the processes of DNA synthesis (replication), RNA synthesis (transcription) and protein synthesis (translation) to not only access, but also to manipulate, the information potential of organismal DNA.

What biochemical process in cells is characterized by formation of an RNA-DNA hybrid?

Name an applied molecular genetic technique that depends on the formation of RNA-DNA hybrids.
What accounts for the biochemical instability of RNA compared to DNA in laboratory research?

**Structure of DNA**
The biochemical basis of duplex stability is determined by base composition, ionic strength, length of duplex. Hydrogen bonds between purines and pyrimidines on opposing strands hold the double helix together. A-T and A-U base pairs are contain 2 hydrogen bonds, whereas G-C base pairs have 3 hydrogen bonds.

In addition to hydrogen bonding between complementary strands, the DNA helix is also stabilized by hydrophobic interactions between adjacent bases on the same strand (Van der Waals interactions). Although these "stacking" interactions do not contribute as much to helix stability as hydrogen bonding, it is important to note that "nearest neighbor" relationships contribute to complex nucleic acid structures such as those found in highly folded RNA.

Transcription factors regulate initiation of RNA synthesis by binding to specific DNA sequences in gene promoters.

Why do you think most transcription factors bind to bases in the major groove of DNA rather than to the minor groove?

What "scientific" fact did Michael Crichton exploit to explain how dinosaurs could be "regenerated" using dinosaur DNA isolated from fossilized mosquitos in his fictional story Jurassic Park, i.e., can you tell what species a DNA fragment comes from by looking at the nucleotide composition?

**Denaturation and Renaturation of Nucleic Acid Polymers**
The relative amount of single- or double-stranded DNA in solution can be experimentally determined using spectrophotometry to measure ultraviolet light absorbance at a wavelength of 260 nanometers (OD260). The aromatic bases in DNA are less accessible to ultraviolet light in the double-stranded, compared to single-stranded form, which creates a measurable difference in the observed OD260. Using this empirical difference in absorbance, it is possible to observe the effect of temperature on DNA structure by monitoring OD260 over a range of temperature range. The temperature at which 50% of the DNA is denatured is called the Tm or transition temperature.

The Transition Temperature (Tm) of a nucleic acid duplex is strongly effected by three factors;

1) Base composition
2) Duplex length
3) Ionic strength of the solution
Two molecular genetic applications where extent of duplex formation is an important consideration are the use of short oligonucleotides in hybridization reactions, and heteroduplex formations between molecules that are less than 100% complementary. The use of homologous, but not identical, DNA molecules in hybridization reactions is common when sequence divergence exists between two genes, for example, across species or among members of a related gene family.

The reverse of nucleic acid denaturation is renaturation, also referred to as reassociation or hybridization. This bimolecular process is most effected by

- temperature
- ionic strength
- molar concentration of the two complementary strands
- reaction time
- denaturing agents such as formamide or urea which lower the Tm
- dextran sulfate which increases the rate of reassociation

**Cot Curves**
The term Cot is used to describe the kinetics of hybridization between two nucleic strands in solution and is defined by the product of [nucleic acid] x (time). Put simply, when the concentration of two complementary strands in a solution is high, then it takes a shorter time for hybridization to occur than it does when one or both of the strands are present at a low concentration. Cot curves plot percent reassociation versus Cot (mole secs/liter), and are used to measure the sequence complexity of DNA samples. DNA from organisms with small genomes have low sequence complexity and reassociate at much lower Cot values, than do denatured DNA samples from more complex organisms.

*What explains the increase in OD260 values of a DNA solution when the temperature is raised from 50 degrees to 90 degrees?*

*Why does adding NaCl to a DNA solution increase the Tm of a DNA duplex?*

*If you wanted to identify a similar, but not identical, gene sequence in a different species using DNA hybridization methods, you could add NaCl to the hybridization reaction to increase your chances of finding the homologous gene. Explain.*
Chemical synthesis of DNA and RNA
Custom-designed oligonucleotides are available commercially and are used routinely in numerous experimental procedures. For example, oligonucleotides are used as template primers in DNA sequencing and PCR reactions, and for the incorporation of site-specific mutations in cloned genes. In addition, chemically-modified ribonucleotides can be used to synthesize large quantities of RNA for use as “antisense” or interference RNA (RNAi) inhibitors of RNA function.

In vitro DNA synthesis reactions take place inside sealed columns that contain glass beads which serve as the solid support for the sequential chemical reactions. Single phosphoramidites for each of the four bases are added to a growing chain that is initiated at the 3’ end.

The five chemical steps required for each nucleotide addition are;

1) **De-blocking** the 5’ end by DMT removal
2) **Activation** of the incoming phosphoramidite
3) **Coupling** of the nucleotides through a 5’-3’ linkage
4) **Capping** of unreacted nucleosides to prevent extension of incomplete products
5) **Oxidizing** the phosphate triester to stabilize the 5’-3’ linkage

**What explains the observation that oligonucleotides of 20 bases or longer can initiate DNA synthesis at sites in a genome that are complementary to only 6-8 nucleotides in the primer under physiological conditions (37 degrees and 100 mM NaCl)?**

**What could you do to increase specificity in the reaction, e.g., to get the primer to work for PCR?**

**Why is high throughput oligonucleotide synthesis so important to applied molecular genetic methods, i.e., what is it about a template primer that makes it so useful?**