OUTLINE - REGULATION ctd:

A) tryptophan biosynthesis in bacteria

*Trp R* codes for a repressor that prevents transcription of a five-polypeptide operon only when it is bound by tryptophan

repression alters mRNA level 70 fold

the *TrpR* promoter is autoregulated by the repressor protein it encodes

Attenuation:

A leader on the trp operon mRNA has two trp codons before a stop codon

If there are plenty of charged trp·tRNAs available, translation of the leader up to the stop codon is rapid and a transcription termination loop forms in the nascent mRNA between the ribosome and the RNA polymerase, causing the polymerase to be released

If the ribosome stalls at a trp codon, an alternative loop prevents the termination loop from forming and transcription proceeds to make the full-length mRNA

attenuation alters expression of the trp operon 10-fold

at least 7 amino acid biosynthetic pathways use attenuation

B) lysis versus lysogeny in Lambda bacteriophage

(see text for details and model)

On entry of λ DNA into a host cell, left and right promoters (PL and PR) are recognized by host О factor and RNA polymerase to read 2 genes, *N* and *Cro*. *N* codes for an antiterminator that allows longer messages that are translated to make the additional proteins CI, CII and CIII. CII, protected from host HflA protein by CIII, acts as a О factor for PE, a promoter for establishment of lysogeny. This results in more CI protein being made. CI is the repressor of the lytic cycle. Cro protein stimulates "outward" expression of genes from PL and PR needed for lysis and lowers "inward" synthesis of CI mRNA by binding to OR3. OR3 is one segment of a 3-part operator (OR3-OR2-OR1) between PL and PR. CI binds preferentially to OR1, preventing outward transcription of lysis genes and enhancing its own synthesis from PM, a promoter for maintenance of lysogeny.

Thus there is a "competition" for entering lysogeny versus the lytic cycle. In most infections, some host cells become lysogenized and thus protected from λ, resulting in cloudy plaques.
If the bacterial cells are "insulted" so that RecA protein is made, it cleaves CI protein and induces the lytic cycle.

C) Regulation of transcription in eukaryotes

Gene expression in higher organisms is often a balance between positive and negative signals; promoters can be complex and include multiple response elements (enhancers and silencers) that are bound by trans-acting factors to stimulate or repress transcription. Interaction between the factors and RNA polymerase involves folding of the DNA double helix.

3 types of protein motifs are known for ability to interact with DNA:

- Zinc fingers
- helix-turn-helix
- leucine zippers

Hormones stimulate expression of specific genes via interaction with receptors

- Steroid hormones such as estrogen, testosterone in animals, gibberillic acid in plants and ecdysone in insects enter the cytoplasm, bind to a receptor (often present only in certain tissues) and the complex enters the nucleus to alter expression.

- Peptide hormones such as NGF, leptin insulin, etc. bind externally to transmembrane proteins and trigger an internal response leading to a "2nd messenger". The signal transduction cascade may involve phosphorylation/dephosphorylation of G-proteins, MAP kinases and other steps.

DNA methylation may be involved in regulation;

- C's next to Gs are often methylated and 'CG' islands are present in most promoters. As a general rule, if the Cs are methylated, the gene is inactive but transcription increases when the Cs are under-methylated. Symmetrical 5-MCG sequences are maintained by maintenance methylases after DNA replication, but site specific methyalses or demthylases that would be required for selective expression are essentially unknown.

Histone removal is associated with gene expression

Histone acetylases and deacetylases lead to remodeling of chromatin structure and affect gene activity.

Hormones/receptors may recruit these enzymes to assist transcription

For both of these models, cause and effect are difficult to unravel.
Post-transcriptional regulation of gene expression

mRNA stability can vary considerably

- longer polyA tails may stabilize
- mRNAs may be stored in eggs for later translation
- hormones that induce expression can stabilize mRNAs
  - vitellogenin mRNA in frog eggs has half-life of 500 hours in the presence of estrogen, 16 hours in its absence
  - casein mRNA in rats mammary glands half-life drops from 92 to 5 hours in absence of prolactin
  - α-amylase mRNA in barley seeds half-life drops from 100 hours to 1/2 hour after heat shock

alternative splicing can result in different proteins from the same pre-mRNA:

- α-tropomysin made from 10 of 13 exons in striated muscle but from only 9 in smooth muscle; only 7 exons used in both types

mRNA editing can occur where a specific base is changed altering a codon

- codon preference or bias can affect rate of translation, slowing when codons that call for rare tRNAs are present

RNAi is a mechanism where short double stranded regions of RNA induce enzymes called dicer and a RISC complex cause mRNAs with a homologous sequence to be effectively ‘chopped up’ and so inactivated. Short foldbacks (hairpins) can be created to target elimination of most any specified mRNA. See [http://www.youtube.com/watch?v=D-77BvL0ld0](http://www.youtube.com/watch?v=D-77BvL0ld0)

protein stability can be altered

- modifications can affect stability
  - ubiquitin targets proteins to be destroyed
    - misfolded, denatured or fragments of proteins targeted
    - destruction catalyzed by a "proteosome" complex using ATP
  - amino acid sequences can alter stability
In yeast, proteins with N terminal arg, lys, phe, leu, or trp have 1/2 life of less than 3 minutes.

N terminal amino acids cys, ala, ser, thr, gly, val, pro or met lead to 1/2 life over 20 hours.

Feedback (endproduct) inhibition can control flux through pathways by allosteric interactions of the end-product of the pathway with the first unique enzyme.