MOLECULAR CELL PHYSIOLOGY - Control of Gene Expression

- **I. What characterizes cell types and what enables an organism to respond to environment?**
  - **A. Key point: The actions and properties of each cell type are determined by the proteins it contains**
  - **A. Types and amounts of various proteins which is regulated by….**
    - **1. Concentration of each protein's corresponding mRNA**
      - **a) Control of gene transcription - differential gene expression**
      - **b) Rate of transcription - initiation most important**
    - **2. Frequency of translation**
    - **3. Protein stability**
- **II. Genes**
  - **A. What is a gene?**
    - **1. A section of DNA which encodes for specific RNAs and subsequently, proteins**
  - **B. What are the different forms of genes?**
    - **1. Alleles - alternate forms of genes**
- **III. Gene control: how are genes expressed?**
  - **A. Prokaryotes - the operon model (Jacob-Monod model)**
    - **1. Regulator gene**
      - **a) Produces a repressor which can interact with the operator**
    - **2. Operator site (the operon)**
      - **a) Promoter - precedes genes and serves as binding site for RNA polymerase**
        - **1) Transcription for promoters is initiated by sigma (σ) factors in RNA polymerase that recognize upstream DNA sequences (within 60 bp of promoter)**
      - **a) Operator - (like allosteric site in enzymes) site for repressor; many include inverted repeats**
      - **c) Activator - allows transcription by preventing repressor binding**
        - **1) Repressors and activators are trans-acting, in that they affect expression on DNA regardless of location**
        - **2) Most bacterial repressors are dimers that demonstrate a helix-turn-helix motif**
        - **1) Cyclic AMP (cAMP), activated by the catabolite activator protein (CAP) provides positive control of the lac operon when glucose levels are low**
    - **3. Structural genes (three inducible proteins)**
    - **4. Mutations**
      - **a) Promoter mutations can be cis-acting, in that they only affect expression on the same DNA molecule in which the mutation occurs**
      - **b) Promoter mutations can decrease (down-mutation) or increase (up-mutation) transcription**
      - **c) Operator mutations that decrease repressor binding (and hence, allow expression) result in constitutive transcription**
  - **A. Analysis of prokaryotic transcription initiation**
    - **1. Footprinting**
      - **a) Protein bound to DNA (such as RNA polymerase), it protects DNA from digestion by DNase**
      - **b) Can use this to find binding region by comparing gels of DNA with and without binding protein after treatment with DNase**
  - **B. Eukaryotes: "The most characteristic and exacting requirement of gene control in multicellular organisms is the execution of precise developmental decisions so that the right gene is activated in the right cell at the right time during development"**
    - **1. Cells do become specialized - they differentiate**
    - **2. Differentiation through selective gene expression**

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3. **Types of gene expression**
   a) **Transcriptional**
      1) **Use nascent-chain analysis (run-on analysis) to evaluate by incubating RNA precursors with nuclei from cell extracts and analyzing for RNA transcripts**
      1) **Cis-acting DNA control elements far away from the promoter (1000s of base pairs away) - enables multiple transcription control factors**
      2) **There are three RNA polymerases in eukaryotes; all three contain multiple units (2 large and 2 small core units with several subunits)**
         a)) **RNA polymerase I synthesizes pre-rRNA, which eventually gets processed to become some of the rRNA subunits**
         b)) **RNA polymerase II synthesizes mRNA and some of the small nuclear mRNAs that participate in mRNA splicing; it can be stimulated by distant enhancer sites and also requires transcription factors such as TFIID – which binds to TATA box**
         c)) **RNA polymerase III synthesizes tRNAs, 5S rRNA, and some other RNAs**
      3) **Transcription factors**
         a)) **Often classified according to the type of DNA-binding domain they contain (examples: homeodomain proteins, zinc-finger proteins, winged-helix proteins, leucine-zipper proteins, helix-loop-helix proteins)**
      4) **Mitochondrial DNA is transcribed by nuclear-encoded RNA polymerase; Chloroplast DNA is transcribed by chloroplast-encoded RNA polymerase**
   b) **Transcript processing (mRNA processing)**
   c) **Transport control (from nucleus to ribosome)**
   a) **Translational**
   b) **Post-translational - protein modification**

IV. **Genetic engineering (a review)**
   A. **Natural recombination**
      1. **Exchange of DNA and recombination of DNA segments**
         A. **Crossing over of chromosomes**
         B. **Recombination of chromosomes pairs**
         C. **Transposition - genes jump from one region of DNA to another**
      2. **Variation of species**
         A. **Humans have 3 billion nucleotides in each of 23 chromosomes**
         B. **There are 2^{23}, or 8,388,608 possible combinations of the 23 chromosomes**
   B. **Recombinant DNA technology - targeted manipulation**
      1. **Genes of interest are isolated**
      2. **Genes are modified**
      3. **Genes are reinserted into the same organism or into a different organism**
   C. **Generalized procedure**
      1. **Focus on a protein of interest**
      2. **Determine the DNA sequence which encodes for that protein**
      3. **Clone the DNA to obtain large supply - use a vector which will "splice in" DNA**
      4. **Allow the vector to integrate its DNA into a host (like E. coli)**
      5. **In mammals, foreign DNA is integrated by microinjection, viruses, etc.**
      6. **Integrated DNA can be used to make bulk protein (insulin)**
      7. **Integrated DNA may or may not alter the organism**