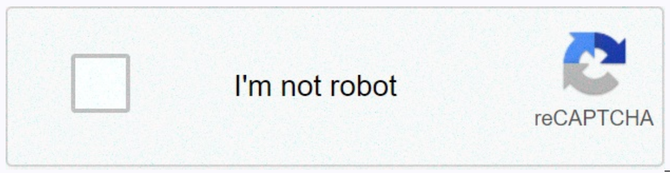


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# Control of gene expression in prokaryotes pogil model 2

Regulation of Gene Expression Cellular function is affected by cellular environment. Adaptation to specific environments is achieved by regulating the expression of genes that encode the enzymes and proteins necessary for survival in a particular environment. Factors that affect gene expression include nutrients, temperature, light, toxins, metals, chemicals and signals from other cells. Malfunctions in regulating gene expression can cause various human disorders and diseases. Regulation in Prokaryotes Bacteria has a simple general mechanism for coordinating the regulation of genes that encode products involved in a set of related processes. The gene cluster and promoter, plus additional sequences that operate together in regulation, are called an operon. The Lactose Operon (lac operon) The lactose operon of E. coli encodes the enzyme b-galactosdact that lactose in galactose and glucose hydrostand. The lac operon contains three cistrons or DNA fragments that encode a functional protein. The proteins encoded by cistrons can function alone or as sub-units of larger enzymes or structural proteins. The Z gene encodes for b-galacosidase. The Y gene encodes a permease that facilitates the transportation of lactose in the bacterium. The A gene encodes a thiogalactoside transacetylase whose function is not known. All three of these genes are transcribed as a single, polympyronic mRNA. Policistronic RNA contains several genetic messages each with its own translation initiation and termination signals. Regulation of the lac Operon The activity of the promoter who controls the expression of the lac operon is regulated by two different proteins. One of the proteins prevents the RNA from transcribing polymerase (negative control), the other increases the binding of RNA polymerase to the promoter (positive control). Negative control of the lac Operon The protein that inhibits transcription of the lac operon is a tetramer with four identical subunits called lac suppressant. The lac suppressor is encoded by the lacI gene, located upstream from the lac operon and has its own promoter. Expression of the lacI gene is not regulated and very low levels of the lac suppressor are constantly synthesized. Genes whose expression is not regulated are called constitutional genes. In the absence of lactose, the lac repressor blocks the expression of the lac operon by binding to the DNS on a website, called the operator that is downstream from the promoter and upstream from the transcription initiation website. The operator consists of a specific nucleotyide sequence recognized by the suppressor that binds very tightly, physically blocking (strangling) the onset of transcription. The lac suppressor has a high affinity for lactose. When a small amount present, the lacquer will bind it that causes dissociation of the DNA operator to for gene expression. Substrates that cause suppressors to disassociate from their operators are called inducers and the genes regulated by such oppressors are called unreadable genes. Positive control of the lac Operon Although lactose can cause the expression of lac operon, the level of expression is very low. The reason is that the lac operon is subject to catabolite suppression or the reduced expression of genes brought on by growth in the presence of glucose. Glucose is very easily metabolized so is the preferred fuel source over lactose, there it makes sense to prevent expression of lac operon when glucose is present. The strength of a promoter is determined by his ability to bind RNA polymerase and form an open complex. The promoter for the lac operon is weak and as a result the lac operon is poorly transcribeed at induction. There is a binding site, upstream from the promoter, for a protein called the catabolite activation protein (CAP). When the CAP protein binds, it distorts the DNA so that the RNA polymerase can bind more effectively, so transcription of the lac operon is greatly improved. In order to bind the CAP must first bind cyclical AMP (cAMP), a second messenger synthesized from ATP by the enzyme Adenylate Cyclase. In the presence of glucose circulating cAMP levels are very low and consequently the onset of transcription of the lac operon is very low. As glucose levels reduce the concentration of cAMP, cap activation increases which in turn binds to the CAP site which stimulates transcription. The cAMP-CAP complex is called a positive regulator. The Arabinose Operon Arabinose is a five-carbon sugar that can serve as energy and carbon source for E. coli. Arabinose should first be converted into ribulose-5 phosphate before it can be metabolized. The arabinose operon has three genes, araB, araA and araD that encode for three enzymes to perform this conversion. A fourth gene, araC, which has its own promoter, encodes a regulatory factor called the C protein. The regulatory sites of the arya operon include four sites that bind the C protein and one CAP binding site. The araO1 and araO2 sites are upstream from the promoter and CAP binding sites. The other two C protein binding sites called araI1 and araI2 are located between the CAP binding site and the promoter. Negative control of the araC Operon In the absence of arabinose, dimators of the C protein bind to araO2, araO1 and araI1. The C proteins bound to associate araO2 and araI1 with each other causing the DNA between them to form a loop effectively blocking transcription of the operon. Positive control of the araC Operon The C protein binds arabinose and undergoes a conformation change that enables it to also bind the and araI2 websites. This leads to the generation of another DNA loop formed by the interaction of C proteins bound to the araO1 araO2 websites. The formation of this loop stimulates transcription of the araC gene resulting in additional C protein synthesis, so the C protein autoregulates its own synthesis. In the absence of glucose, cAMP-CAP is formed which binds to the CAP website. C protein bound at the araI1 and araI2 sites interact with the bound CAP allowing RNA to initiate polymerase transcription of the arya operon promoter. The Tryptophan Operon E. coli can synthesize all 20 of the natural amino acids. Amino acid synthesis consumes a lot of energy, to prevent the waste of energy from tightly regulated the operons that encode for amino acid synthesis. The trp operon consists of five genes, trpE, trpD, trpC, trpB and trpA, which encode for the enzymes necessary for the synthesis of tryptophan. The trp operon is regulated by two mechanisms, negative nuclear power and weakening. Most of the operons involved in amino acid synthesis are regulated by these two mechanisms. Negative Corepression The trp operon is negatively controlled by the trp repressor, a product of the trpR gene. The trp repressor binds to the operator and blocks transcription of the operon. However, to bind to the operator, the suppressor must first bind to Trp, therefore tryptophan is a core pressor. In the absence of Trp, the trp suppressor dissociates and transcription of the trp operon is initiated. Weakening Attenuation regulates termination of transcription as a function of tryptophan concentration. At low levels of trp full-length mRNA made, at high levels transcript of the trp operon are prematurely stopped. Weakening works by connecting transcript to translation. Prokaryotic mRNA does not require processing and since prokaryotes cannot start any core translation of mRNA before transcription is complete. Consequently, regulating gene expression via weakening is unique to prokaryotes. A. Deterioration is mediated by the formation of one of two possible stem loop structures in a 5' segment of the trp operon in the mRNA. B. If tryptophan concentrations are low then translation of the leader peptide is slow and transcription of the trp operon surpasses translation. This leads to the formation of a non-alternating stem loop structure between regions 2 and 3 in the 5' segment of the mRNA. Transcript of the trp operon is then completed. c. If tryptophan concentrations are high the ribosome quickly translate the mRNA leader peptide. Because translation occurs quickly, the riboon region covers 2 so that it cannot attach to region 3. Consequently, the formation of a tribal loop structure between regions 3 and 4 farm and transcription is terminated. Regulation of Gene Expression in Eukaryotes The genetic information of a human cell is a thousand folding larger than that of a prokaryotic cell. Things are further complicated by the number of cell types and the fact that seltipe seltipe expresses a particular subset of genes at different points in an organism development. Regulating gene expression so that a particular subset of genes in a specific tissue is expressed at specific points of development is very complicated. This increased complexity in regulation lends itself to malfunctions that cause disease. Three ways eukarotes regulate gene expression will be discussed: change of gene content or position, transcription regulation and alternative RNA processing. 1. Change of Gene Content or Position The copy number of a gene or its location on the chromosome can significantly effect its level of expression. No content or location can be changed by gene reduction, shredding, or rearrangement. Gene Amplification The expression of a specific gene can be supplemented by enhanceing the copy number. Histone proteins and rRNA are needed in large quantities by almost all eukaryotic cells therefore the genes encoding histones and rRNA exist in a permanently fortified state. Gene starocation can present problems with the use of chemotherapeutic drugs. Methotrexate inhibits dihydrofolate reductions, the enzyme responsible for regenerating the folate used in nucleotide synthesis. Tumor cells often become resistant to the drug, because the gene encoding dihydrofolate reductions are reinforced by several hundred folding leading to more enzyme production then can handle the drug. Gene Diminution A gene whose expression is required only at a particular development point or in a particular tissue can be turned off by no shredding. Since reticulocytes mature into red blood cells, all their genes are lost because the core is degraded. Gene Rearrangements No rearrangement is used to generate each of the genes that code the millions of different antibodies produced by B cells. Sometimes bad no rearranges occur leading to improper gene regulation. It often occurs in cancer cells. Translocation of a segment of chromosome 8 to chromosomes that encode immunoglobulins leads to activation of a gene that converts healthy B cells into Burkitt's lymphoma cells (unregulated proliferator B cells). 2. Transcriptional regulation By Chromosomal Packaging regions of each of the different chromosomes is packaged either as heterochromatein or euchromatoin. In heterochromatin, the DNA is very tightly condensed and inaccessibley delivered to the transcriptional machinery, as a result heterochromatin is transcriptically inactive. In human women, one of each of the two X chromosomes is completely activated by being packed into a heterochromatin to form a Barr body. The Cys remains in DNA in the heterochromatin are heavily methylated suggesting that methylation may play a role in the of heterochromatin. Drugs that interfere with methylation cause activation of previously inactive genes found in heterochromatin. heterochromatin. euchromatin the DNA is not so condensed and is accessible to the transcription machinery. The regions of a chromosome maintained as hetero- and eu chromateine can vary in a specific manner. This can enable the cells of specific tissues to express a particular subset of genes necessary for tissue function. By Individual Gene Trans-acting Elements Proteins that participate in regulating gene expression are often called trans acting elements. At least 100 different proteins, very specific to the regulation of a particular gene, are known. Others play a more common role in regulating gene expression in a manner that is analogous to activating numerous prokaryotic genes through the CAP-cAMP complex. Trans-acting factors have multiple domains necessary for activity and can include DNS binding, transcriptional activation, and ligand-binding domains. DNS Binding Domains DNS binding domains recognize specific DNS sequents in the regulatory regions of a gene. The DNA-binding domains of a regulatory protein generally consist of one of three motives: helix-turn-helix, zinc finger or leucine zipper. DNA-binding proteins that possess these motives bind with high affinity to their recognition sites and with low affinity to other DNA. A very small portion of the protein makes contact with the DNA through H effects and van der Waals interactions between amino acid side chains and the functional groups in the large groove and the phosphate backbone of the DNA. The rest of the protein is involved in proper positioning of the DNA-binding domain and making protein protein contacts with other transcription proteins. The Helix-Turn-Helix Motif Proteins with this motif form symmetrical dimerders that recognize a symmetrical palindrome DNA sequence. Each monomer of the dimer contains a region in which two a helicas are kept together at 90 degrees by a turn of four amino acids. One set of helicates makes contact with about five base pairs in the large groove. The other set sits atop the phosphate spine and helps to properly position the set of helices that fit into the large groove. The Zinc Finger Motive Proteins that own this motive contains between 2 to 9 repeat domains each centered on a tetrahedrally coordinated sinkion. Each zinc coordinated domain forms a loop with an a-helix, this loop is called a zinc finger. There are two types of zinc fingers: the C2H2 finger and the Cx finger. C2H2 Finger: Three fingers interact with the large groove and wrap around the DNA. Many transcription factors have this type of domain. Cx Finger: Protein with this motif binds as dimers to the great groove of the DNA. Many steroid receptors have this type of domain. The Leucine Zipper Motif Proteins with this type of motif have an amphipathic a-helix at their carboxyl One side of the helix consists of hydrophobic groups, usually leucine, that repeat each seventh position for multiple turns of the glories. The other face consists of charged and polar groups. Proteins with this motif bind as dimers to the large groove of the DNA. The two a-helices of each arm enter the large groove and wrap around the double henal. Several oncogenes use this type of motive. Transcription-Entit domains These domains generally act separately and independently of the DNS binding domains. Transcription-entving domains improve transcription by being physically infallible with other regulatory proteins and/or with RNA polymerase. The actual mechanisms by which these domains enable or improve transcription are not known. Ligand-Binding Domains Steroid Hormones, Thyroid Hormones and Retinoic Acid are examples of ligands that enable transcription by binding to a specific domain on a receptor protein. When binding the receptor, a conformation undergoes change that enables it to bind DNA. Once bound to the DNA can trigger a receptor protein or suppress transcription of the target gene. Cis-acting Elements Cis acting elements are DNA sequents that are recognized and bound by the trans-acting elements that regulate transcription. There are two large types of cis-acting elements: promoters and regulatory elements. Promoters Promoters are the sites where RNA should bind polymerase to the DNA to initiate transcription (see RNA Synthesis and Processing lecture). The rate or efficiency of promoter use by RNA polymerase is affected by the regulatory elements. Regulatory Elements Regulatory elements are specific DNS sequents that are recognized and bound by the trans-acting elements that stimulate or inhibit the expression of a particular gene. There are two types: amplifiers and response elements. Enhancers are regulatory elements that increase or suppress the rate of no transcript. Response elements are regulatory sequents that facilitate the coordinated regulation of a group of genes. Certain ligands like steroid hormones and cAMP bind to their receptors that in turn bind to activate their response element or inhibit transcription. 3. Alternative processing alternatives starting sites initiating transcription at an alternative starting site posting another exon at the 5' end of the transcript. Examples of genes using alternative starting sites as a form of regulation include amylase, mysine and alcohol dehydrogenase. Alternative Polygylation Websites Immunoglobin (antibody) heavy chains use an alternative polygylation website to affect the length of transcripts. The longer transcription encodes the mm shape that is localized to the cell membranes of lymphocytes, the shorter transcription encodes the secreted form, Ms. Merkel's lymphocy. Alternative Splice Sites Alternative splice sites are used to to generate proteins with tissue specific functions called isoforms. Many peptide hormones exist as isoforms such as the gene that produces differentially spliced calcium in the thyroid gland and calcitonin gene-related peptide in the neurons. Regulation of mRNA Stability The stability of mRNA is fairly variable shape gene too none. These variations in stability govern the length of time that mRNA is available for translation and thus the amount of protein synthesized. The half-steps of mRNA range from 10 hours to minutes. Sequences in the 3'untranslated region of mRNA that serve as signals for rapid deterioration have been identified in some mRNA's with very short half-lending. The length of the poly a tail also affects mRNA stability, with longer tails trending to have longer half-sleds. 