

1. Crisprs Stands For?

Clustered Regularly Interspaced Short Palindromic Repeats (or CRISPRs)

2. Operon Model and Five Feature?

Groups of genes coding for related proteins are arranged in units known as operons. An operon consists of an operator, promoter, regulator, and structural genes. The regulator gene codes for a repressor protein that binds to the operator, obstructing the promoter (thus, transcription) of the structural genes.

1. The products of the *lacZ*, *lacY* and *lacA* genes are encoded in a single polycistronic *lac* mRNA molecule.
2. The promoter for this mRNA molecule is immediately adjacent to the *lac* region.
3. The operator is a sequence of bases (in the DNA) to which the repressor protein binds.
4. When the repressor protein is bound to the operator, *lac* mRNA transcription can't take place.
5. Inducers stimulate *lac* mRNA synthesis by binding to the repressor. This binding alters the repressor's conformation so it can't bind to the operator. In the presence of an inducer, therefore, The operator is unoccupied and the promoter is available for initiation of mRNA synthesis. This state is called derepression.

3. Name 2 Proteins Used In E.Coli

One, the *lac* repressor, acts as a lactose sensor.

The other, catabolite activator protein (CAP), acts as a glucose sensor.

4. Torpedo Model?

An enzyme that degrades the second RNA as it emerges from the polymerase has been identified, and this enzyme may itself trigger termination. This is called the torpedo model of termination.

5. Involvement of CTD Tail In Transcription?

RNA polymerase II **transcription** elongation and Pol II **CTD** Ser2 phosphorylation: **Atail** of two kinases. Author information: ... The transition between initiation and productive elongation during RNA Polymerase II (Pol II) **transcription** is a well-appreciated point of regulation across many eukaryotes.

6. Two Protein Function And Name?

Lac sensor

CAP catabolite

7. How Short life span of Mrna analysis Which Two Experiment Experiment Name (1) Experiment Explanation (2) ?

The half life of a typical bacterial mRNA is a few minutes. The particular mRNA and their life span can be analyzed by the pulse-chase experiment. Bacteria are briefly cultured in a medium that contains a radioactive precursor for RNA such as [³H] uridine. Then the bacteria are switched to a medium that contains a high concentration of non radioactive uridine while no [³H] uridine and samples are removed at specific times for analyses. The RNA are isolated and different types are separated by gel electrophoresis and detected by their radioactivity. A

typical radioactive mRNA molecule will decrease with a half life of a few minutes, whereas radioactive rRNA and tRNA will remain through many generations. However, bacteria also contain some longlived mRNA molecules which can not be analyzed by this technique.

1st Experiment Name:

Pulse Chase

2nd Experiment Pulse Chase:

Pulse chase experiment, mRNA synthesized during a short time window was labelled. These studies showed that mRNA is synthesized in the nucleus. Within an hour, most of this RNA had left the nucleus and was observed in the end cytoplasm.

8. How Many CTD Heptatidase In Repeat Sequence In Yeast Drosophila And Human?

There are 27 of these repeats in the yeast Pol II CTD, 45 in the fly Drosophila, and 52 in humans.

9. What ADAR? 3 Gene Close to Human ADAR?

Double-stranded RNA-specific adenosine deaminase is an enzyme that in humans is encoded by the **ADAR** gene (which stands for adenosine deaminase acting on RNA). ... The conversion from A to I in the RNA disrupt the normal A:U pairing which makes the RNA unstable.

Humans and mice contain three ADAR genes: ADAR1, ADAR2, and ADAR3.

10. RNA Elongation In Three Lines?

elongation is the stage when the **RNA** strand gets longer, thanks to the addition of new nucleotides. During **elongation**, **RNA** polymerase "walks" along one strand of DNA, known as the template strand, in the 3' to 5' direction.

11. How Regulatory Protein Control Gene Expression?

Regulated stages of gene expression. Any step of gene expression may be modulated, from the DNA-RNA transcription step to post-translational modification of a protein. The following is a list of stages where gene expression is regulated, the most extensively utilised point is Transcription

12. Write Point Operon?

an **operon** is a functioning unit of DNA containing a cluster of genes under the control of a single promoter

13. Name Two Protein Which Is Present In E. Coli?

lac catabolite activator protein

14. RNA Polymerase I And 3 Function?

RNA polymerase I (RNAPI) transcribes **rRNA** genes, RNA polymerase II (RNAPII) transcribes **mRNA**, miRNA, snRNA, and snoRNA genes, and RNA polymerase III (RNAPIII) transcribes **tRNA** and 5S **rRNA** genes. This is in contrast with prokaryotes where a single RNA polymerase is responsible for the transcription of all genes.

15. Requirement For Transcription?

important is the concept that **transcription**, whether prokaryotic or eukaryotic, has three main events. Initiation - binding of RNA polymerase to double-stranded DNA; this step involves a transition to single-strandedness in the region of binding; RNA polymerase binds at a sequence of DNA called the promoter.

16. Splicing?

splicing is the editing of a newly made precursor messenger RNA (pre-mRNA) transcript into a mature messenger RNA (mRNA). After **splicing**, introns are removed and exons are joined together.

17. Gene Expression?

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product.

18. CRISPR and Application?

CRISPR technology is a simple yet powerful tool for editing genomes. It allows researchers to easily alter DNA sequences and modify gene function. Its many potential applications include correcting genetic defects, treating and preventing the spread of diseases and improving crops

19. Plus chase experiment, to verify life short span of mRNA?

Pulse chase" experiment, mRNA synthesized during a short time window was labelled. These studies showed that mRNA is synthesized in the nucleus. Within an hour, most of this RNA had left the nucleus and was observed in the end cytoplasm.

20. Define Termination Sequence?

Once the polymerase has transcribed the length of the gene (or genes), it must stop and release the RNA product (as well as dissociating from the DNA itself). This step is called termination.

21. Write The Process Of Elongating Polymerase?

The Elongating Polymerase Is a processive machine that synthesizes and proofreads RNA. DNA passes through the elongating enzyme in a manner very similar to its passage through the open complex.

Thus, doublestranded DNA enters the front of the enzyme between the pincers. At the opening of the catalytic cleft, the strands separate to follow different paths through the enzyme. The strands exit via their respective channels and re-form a double helix behind the elongating polymerase.

22. Five Steps Of Operon Model?

1. The products of the lacZ, lacY and lacA genes are encoded in a single polycistronic lac mRNA molecule.
2. The promoter for this mRNA molecule is immediately adjacent to the lac region.
3. The operator is a sequence of bases (in the DNA) to which the repressor protein binds.
4. When the repressor protein is bound to the operator, lac mRNA transcription can't take place.
5. Inducers stimulate lac mRNA synthesis by binding to the repressor. This binding alters the repressor's conformation so it can't bind to the operator. In the presence of an inducer,

therefore, The operator is unoccupied and the promoter is available for initiation of mRNA synthesis. This state is called derepression.

23. Polarity Of DNA Helicase?

Polarity is ascertained by identifying the duplex region that is denatured by the helicase. For helicases involved in DNA replication, the polarity of the reaction is strongly indicative of helicase placement on the leading (a 3' -5' polarity) or lagging (a 5' +3' polarity) strand.

24. Function Of CPD Photolyase?

Increase in CPD photolyase activity functions effectively to prevent growth inhibition caused by UVB radiation. ... Conversely, the AS-D plant had little photolyase activity, was severely damaged by elevated UVB radiation, and maintained higher CPD levels in its leaves during growth under UVB radiation.

25. Advancement Of Molecular Bio In Agriculture Science?

Agricultural molecular is a collection of scientific techniques used to improve plants, animals and microorganisms. Based on an structure and characteristics of DNA, scientists have developed solutions to increase agricultural productivity. Scientists have learned how to move genes from one organism to another. This has been called genetic modification (GM), genetic engineering (GE) or genetic improvement (GI).

26. Standard Amino Acids 5 Name?

Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, And Valine

27. 2 Mutations Which Cause One Base Change?

The simplest mutations are switches of one base for another. There are two kinds of such mutations which include:- Transitions Transversions

28. Types Of UV Light?

Ultraviolet light is divided into three bands: UV -A (321-400nm) UV -B (296-320nm) UV -C (100-295nm)

29. Role Of Regulatory Proteins In Gene Expression?

Genes are very often controlled by extracellular signals; in the case of bacteria, these signals typically mean molecules present in the growth medium. These signals are communicated to genes by regulatory proteins, which come in two types: positive regulators, or activators, and negative regulators, or repressors. Typically, these regulators are DNA binding proteins that recognize specific sites at or near the genes they control. An activator increases transcription of the regulated gene, and repressors decrease or eliminate that transcription.

30. Splicing and Classes?

Splicing is the editing of a newly made precursor messenger RNA Exons, introns, alternative splicing.

31. Spliceosome?

A spliceosome is a large and complex molecular machine found primarily within the nucleus of eukaryotic cells. The spliceosome is assembled from snRNAs and approximately 80 proteins

32. Lactose metabolism?

Metabolism. Your body starts metabolizing or breaking down lactose in the intestines with the help of lactase, an enzyme produced and released by cells that line your small intestine. The enzyme attaches to lactose and cleaves it into molecules of galactose and glucose

33. Transition work with open complex?

Transition to the Open Complex involves structural changes in RNA Polymerase and in the Promoter DNA. The initial binding of RNA polymerase to the promoter DNA in the closed complex leaves the DNA in double-stranded form. The transition from the closed to the open complex involves structural changes in the enzyme and the opening of the DNA double helix to reveal the template and nontemplate strand

34. Half Life of mRNA?

In prokaryotes, which lack a cell nucleus, transcription and translation occur nearly simultaneously. Prokaryotic mRNAs have very short half-lives (with an average half-life of 3 minutes) and are usually rapidly degraded by exonucleases that start at the 3' end and move towards the 5' end.

Operon model?

- Monod and Jacob proposed the operon model in 1961 to explain how the lac system is regulated. The term operon refers to two or more contiguous genes and the genetic elements that regulate their transcription in a coordinate fashion. Promoters had not yet been discovered when Monod & Jacob proposed the operon model but were readily incorporated into the operon model after their discovery.
- The five major features of the model are:-
 1. The products of the *lacZ*, *lacY* and *lacA* genes are encoded in a single polycistronic *lac* mRNA molecule.
 2. The promoter for this mRNA molecule is immediately adjacent to the *lac* region.
- Promoter mutations (p-) that are completely incapable of making β -galactosidase, permease, and transacetylase have been isolated. The promoter is located between *lacI* and *lacO*.
- 3. The operator is a sequence of bases (in the DNA) to which the repressor protein binds.
- 4. When the repressor protein is bound to the operator, *lac* mRNA transcription can't take place.
- 5. Inducers stimulate *lac* mRNA synthesis by binding to the repressor. This binding alters the repressor's conformation so it can't bind to the operator. In the presence of an inducer, therefore,
- The operator is unoccupied and the promoter is available for initiation of mRNA synthesis. This state is called derepression.
- This simple model explains many of the features of the *lac* system and of other negatively regulated genetic systems.
- However, *lac* operon is also subjected to positive regulation.

RNA editing explains?

RNA editing is a molecular process through which some cells can make discrete changes to specific nucleotide sequences within a RNA molecule after it has been generated by RNA polymerase. RNA editing is relatively rare, and common forms of RNA processing (e.g. splicing, 5'-capping and 3'-polyadenylation) are not usually included as editing. Editing events may include the insertion, deletion, and base substitution of nucleotides within the edited RNA molecule.

RNA editing has been observed in some tRNA, rRNA, mRNA or miRNA molecules of eukaryotes and their viruses, archaea and prokaryotes.^[1] RNA editing occurs in the cell nucleus and cytosol, as well as within mitochondria and plastids. In vertebrates, editing is rare and usually consists of a small number of changes to the sequence of affected molecules. In other organisms, extensive editing (*pan-editing*) can occur; in some cases the majority of nucleotides in a mRNA sequence may result from editing.

RNA-editing processes show great molecular diversity, and some appear to be evolutionarily recent achievements that arose independently. The diversity of RNA editing phenomena includes nucleobase modifications such as cytidine (C) to uridine (U) and adenosine (A) to inosine (I) deaminations, as well as non-templated nucleotide additions and insertions. RNA editing in mRNAs effectively alters the amino acid sequence of the encoded protein so that it differs from that predicted by the genomic DNA sequence.

Editing by insertion or deletion

RNA editing through the addition and deletion of uracil has been found in kinetoplasts from the mitochondria of *Trypanosoma brucei*.^[3] Because this may involve a large fraction of the sites in a gene, it is sometimes called "pan-editing" to distinguish it from topical editing of one or a few sites. Pan-editing starts with the base-pairing of the unedited primary transcript with a guide RNA (gRNA), which contains complementary sequences to the regions around the insertion/deletion points. The newly formed double-stranded region is then enveloped by an editosome, a large multi-protein complex that catalyzes the editing.^{[4][5]} The editosome opens the transcript at the first mismatched nucleotide and starts inserting uridines. The inserted uridines will base-pair with the guide RNA, and insertion will continue as long as A or G is present in the guide RNA and will stop when a C or U is encountered.^{[6][7]} The inserted nucleotides cause a frameshift and result in a translated protein that differs from its gene.

The Effect of Uracil Insertion in pre-mRNA transcripts

The mechanism of the editosome involves an endonucleolytic cut at the mismatch point between the guide RNA and the unedited transcript. The next step is catalyzed by one of the enzymes in the complex, a terminal U-transferase, which adds Us from UTP at the 3' end of the mRNA.^[8] The opened ends are held in place by other proteins in the complex. Another

enzyme, a U-specific exoribonuclease, removes the unpaired Us. After editing has made mRNA complementary to gRNA, an RNA ligase rejoins the ends of the edited mRNA transcript.^{[9][10]} As a consequence, the editosome can edit only in a 3' to 5' direction along the primary RNA transcript. The complex can act on only a single guide RNA at a time. Therefore, a RNA transcript requiring extensive editing will need more than one guide RNA and editosome complex

What is CRISPRs? work? application?

Clustered regularly interspaced short palindromic repeats (CRISPR, pronounced *crisper*^[2]) are segments of prokaryotic DNA containing short, repetitive base sequences. In a palindromic repeat, the sequence of nucleotides is the same in both directions. Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA (e.g., a virus or plasmid).^[3] Small clusters of *cas* (CRISPR-associated system) genes are located next to CRISPR sequences.

The CRISPR/Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as those present within plasmids and phages^{[4][5][6]} that provides a form of acquired immunity. RNA covering the spacer sequence helps Cas proteins recognize and cut exogenous DNA. Other RNA-guided Cas proteins cut foreign RNA.^[7] CRISPRs are found in approximately 40% of sequenced bacterial genomes and 90% of sequenced archaea.^{[8][note 1]}

A simple version of the CRISPR/Cas system, CRISPR/Cas9, has been modified to edit genomes. By delivering the Cas9 nuclease complexed with a synthetic guide RNA (gRNA) into a cell, the cell's genome can be cut at a desired location, allowing existing genes to be removed and/or new ones added.^{[9][10][11]} The Cas9-gRNA complex corresponds with the CAS III crRNA complex in the above diagram.

CRISPR/Cas genome editing techniques have many potential applications, including medicine and crop seed enhancement. The use of CRISPR/Cas9-gRNA complex for genome editing^{[12][13]} was the AAAS's choice for breakthrough of the year in 2015.^[14] Bioethical concerns have been raised about the prospect of using CRISPR for germline editing.^[15]

7 Applications

- 7.1 Genome engineering, 7.2 Knockdown/activation, 7.3 RNA editing, 7.4 Disease models, 7.5 Gene drive, 7.6 Biomedicine, 7.7 Gene function, 7.8 In vitro genetic depletion

CRISPR stands for?

- Clustered Regularly Interspaced Short Palindromic Repeats (or CRISPRs).

mRNA has short life span write experiment?

The half life of a typical bacterial mRNA is a few minutes. The particular mRNA and their life span can be analyzed by the pulse-chase experiment. Bacteria are briefly cultured in a medium that contains a radioactive precursor for RNA such as [^3H] uridine. Then the bacteria are switched to a medium that contains a high concentration of non radioactive uridine while no [^3H] uridine and samples are removed at specific times for analyses. The RNA are isolated and different types are separated by gel electrophoresis and detected by their radioactivity. A typical radioactive mRNA molecule will decrease with a half life of a few minutes, whereas radioactive rRNA and tRNA will remain through many generations. However, bacteria also contain some longlived mRNA molecules which can not be analyzed by this technique.

Gene expression controlled by regulatory proteins?

Genes are very often controlled by extracellular signals; in the case of bacteria, these signals typically mean molecules present in the growth medium. These signals are communicated to genes by regulatory proteins, which come in two types: positive regulators, or activators, and negative regulators, or repressors. Typically, these regulators are DNA-binding proteins that recognize specific sites at or near the genes they control. An activator increases transcription of the regulated gene, and repressors decrease or eliminate that transcription. How do these regulators work? First, RNA polymerase binds to the promoter in a closed complex (in which the DNA strands remain together). The polymerase–promoter complex then undergoes a transition to an open complex in which the DNA at the start site of transcription is unwound and the polymerase is positioned to initiate transcription. This is followed by promoter escape, the step in which polymerase leaves the promoter and starts transcribing. Polymerase then proceeds through the elongation phase before finally terminating. Which steps are stimulated by activators and inhibited by repressors depends on the promoter and regulators in question. The most common step at which gene expression is regulated is the initiation of transcription. There are two reasons why this might make sense. First, transcription initiation is the most energetically efficient step to regulate. By this we mean that deciding whether or not to express a gene at the first step ensures that no energy or resources are wasted. Second, regulation at this first step is easier to do. There is only a single copy of each gene and so typically only a single promoter on a single DNA molecule must be regulated to control expression of a given gene. Why then is not all regulation focused on the step of transcription initiation? Regulating later steps can have two advantages. First, it allows for more inputs: if a gene is regulated at more than one step, more signals can modulate its expression, or the same signals can do so even more effectively. Second, regulation at steps later than transcription initiation can reduce the response time. Thus, consider again the example of translational regulation. If a signal relieves repression of this step, the protein product encoded by the gene will be produced immediately upon receipt of that signal. This reduced response time might obviously be advantageous in some situations. Many promoters are regulated by activators that help RNA polymerase bind DNA and by repressors that block that binding. At many promoters, in the absence of regulatory proteins, RNA polymerase binds only weakly. This is because one or

more of the promoter elements is absent or imperfect. When polymerase does occasionally bind, however, it spontaneously undergoes a transition to the open complex and initiates transcription. This gives a low level of constitutive expression called the basal level. Binding of RNA polymerase is the rate-limiting step in this case. To control expression from such a promoter, a repressor need only bind to a site overlapping the region bound by polymerase. In that way, the repressor blocks polymerase binding to the promoter, thereby preventing transcription, although it is important to note that repression can work in other ways as well. The site on DNA where a repressor binds is called an operator. To activate transcription from this promoter, an activator can just help the polymerase bind the promoter. Typically, this is achieved as follows:

The activator uses one surface to bind to a site on the DNA near the promoter; with another surface, the activator simultaneously interacts with RNA polymerase, bringing the enzyme to the promoter. This mechanism, often called recruitment, is an example of cooperative binding of proteins to DNA. The interactions between the activator and polymerase, and between activator and DNA, serve merely “adhesive” roles. The enzyme is active and the activator simply brings it to the nearby promoter. Once there, it spontaneously isomerizes to the open complex and initiates transcription.

Spliceosome:

A **spliceosome** is a large and complex molecular machine found primarily within the splicing speckles of the cell nucleus of eukaryotic cells. The spliceosome is assembled from snRNAs and protein complexes. The spliceosome removes introns from a transcribed pre-mRNA, a type of primary transcript. This process is generally referred to as splicing.^[1] Only eukaryotes have spliceosomes and some organisms have a second spliceosome, the minor spliceosome.^[2] An analogy is a film editor, who selectively cuts out irrelevant or incorrect material (equivalent to the introns) from the dailies and sends the cleaned-up version to be screened for the produce

RNA splicing:

The coding sequence of a gene is a series of three nucleotide codons that specifies the linear sequence of amino acids in its polypeptide product. It is generally assumed that the coding sequence is contiguous; i.e., the codon for one amino acid is immediately adjacent to the codon for the next amino acid in the polypeptide chain. This is true in the vast majority of cases in bacteria and their phage. But it is rarely so for eukaryotic genes. In those cases, the coding sequence is interrupted by stretches of non-coding sequences. Many eukaryotic genes are thus mosaics, consisting of blocks of coding sequences separated from each other by blocks of non-coding sequences. The coding sequences are called **exons** and the intervening sequences are called **introns**. Once transcribed into an RNA transcript, the introns must be removed and the exons joined together to create the mRNA for that gene. The number of introns found within a gene varies enormously—from one in the case of most yeast genes (and a few human genes), to 50 in the case of the chicken $\alpha 2$ collagen gene to as many as 363 in the case of the Titin gene of humans. The sizes of the exons and introns vary as well. Indeed, introns are very often much

longer than the exons they separate. Thus, for example, exons are typically on the order of 150 nucleotides, whereas introns—although they too can be short—can be as long as 800,000 nucleotides (800 kb). As another example, the mammalian gene for the enzyme dihydrofolate reductase is more than 31 kb long, and within it are dispersed six exons that correspond to 2 kb of mRNA. Thus, in this case, the coding portion of the gene is 10% of its total length. Like the uninterrupted genes of prokaryotes, the split genes of eukaryotes are transcribed into a single RNA copy of the entire gene - the primary transcript that contains introns as well as exons. Because the length and number of introns, the primary transcript (or pre-mRNA) can be very long indeed. The process of intron removal is called **RNA Splicing**. It converts the pre-mRNA into mature mRNA containing only exons. RNA Splicing must occur with great precision to avoid the loss, or addition, of even a single nucleotide at the sites at which the exons are joined. The triplet-nucleotide codons of mRNA are translated in a fixed reading frame that is set by the first codon. Lack of precision in splicing will change the reading frames of exons. Some pre-mRNAs can be spliced in more than one way. Thus, mRNAs containing different selections of exons can be generated from a given pre-mRNA. Alternative splicing strategy enables a gene to give rise to more than one polypeptide product. These alternative products are called isoforms. It is estimated that 90% or more of the protein-coding genes in the human genome are spliced in alternative ways to generate more than one isoform.

Describe RNA processing event?

Initiator tRNA (containing methionine) binds to small ribosomal subunit at the P site along with translation initiation factors. unit binds to 5' end of mRNA looking for AUG, initiation factors dissociate when AUG is found large ribosomal subunit assembles. tRNA with next amino acid binds to the large ribosomal subunit at the A site. Initiator tRNA separated from methionine at P site by breaking of energy bonds. C terminal of methionine binds to N terminal of new amino acid using enzyme peptidyl transferase (part of the ribosome). large subunit moves relative to the small subunit (which is still reading the mRNA) so that the tRNA with the newly bound amino acid shifts to the P site small subunit moves 3 nucleotides along mRNA to read next codon. new tRNA carrying the next amino acid is bound to A site and process from step 4 to 8 repeats. when small ribosomal subunit reads stop codons UAA, UAG or UGA, release factors bind to the A site. release factors alter the activity of peptidyl transferase so that water is catalyzed instead of peptide bond. C terminal of newly-formed protein is released into the cytoplasm where it is folded with, or without, the help of chaperone proteins

Some simple notes about translation:

- mRNA always complementary to template strand of DNA and same sequence as coding strand of DNA save for switching T for U. It is read from 5' to 3'.
- tRNA anticodons are complementary to mRNA codons, same as the template strand DNA nucleotides save for switching T for U, and is complementary to the coding strand of DNA

Since you just asked about RNA processing, I'm assuming that you don't need me to go over transcription in detail as well. Essentially, transcription is just RNA polymerase making mRNA that is complementary to the template strand of the DNA. The mRNA has introns that are spiced out, then are capped and added with a poly-A tail. The final product is what gets through the nuclear pores and into the ribosome.

BIO 302