Review Article

Regulation of Gene Expression in Prokaryotes And Eukaryotes

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ABSTRACT

The mechanism which stimulates the expression of certain genes and inhibits that of other is called regulation of gene expression. It is possible only if the organism has a mechanism of regulating gene activity by allowing some to function and others to restrain their activity through switching on and switching off system. This means, the genes are turned on or off as per requirement. A set of genes is switched on when enzymes are required to metabolise a new substrate. The enzymes produced by these genes metabolise the substrate. The molecules of metabolite that come to switch on of the genes are termed as inducers and the phenomenon is called induction. Certain genes continue to express themselves till the end product of inhibits or repress their expression. Inhibition by an end product is known as feedback repression. In eukaryotes the gene expression could be expressed at transcriptional level, processing level, translocation of mRNA and translational level.

Key words: *Gene expression, Regulation, Acetylation, Phosphorylation, Methylation*

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Article Info: Date received: 8 Jan. 2019 Date accepted: 22 Mar. 2019

INTRODUCTION

Gene expression is the process by which nucleotide the genetic code of a gene is utilised for protein synthesis and cell structure production. Transcription and translation the two main steps involve in

The process of gene expression- RNA synthesis steps is taken place in transcription process[1]. Translation process involve in formation of amino acid sequences. After gene expression, gene regulation is also needed for controlling rate and manner of gene expression. The on–off of transcription process is the main regulatory control of the gene expression in prokaryotes whereas, more complex regulatory mechanism of transcription takes place in eukaryotes[10].

Gene regulation mechanism include the: regulation of rate of transcription, processing of RNA molecule, regulate the stability of mRNA molecules, regulating the rate of translation. Post- transcriptional and post-translational regulation mechanism also

applied after complete synthesized of gene. There are four identifiable steps during transcription: promoter recognition, chain initiation, chain elongation, chain termination. Promoter plays a vital role in the initiation of transcription of a particular gene.[11] to the promoter sequence RNA polymerase binds, which performs the transcription process, and then beings to work, constructing RNA to match the DNA nucleotides over which the enzyme passes. Inhibitor has also an essential role to prevent the expression of a certain gene. Inhibitors is often used in research either transcription or translation process. During gene expression regulation process occur in two pathways: positive and negative regulation. In human DNA exist in methylated form. Loss of transcriptional activity occur in methylation and inactivation of gene occur. DNA demethylation is also occur along with methylation in which gene promoter is linked to transcriptional activation and gene expression[24]. Histone acetylation and deacetylation, phosphorylation, Dephosphorylation is the further essential steps for gene expression regulation.

Proteins are coded by thousand of gene in cell, it is not necessary that every gene actively producing proteins at all the time. In this process we will study some of the factors which may regulate the gene expression and gene regulation, whenever the gene is in active state. To be expression gene, gene are transcribed into mRNA to protein and the protein become functional. Gene expression occur at any step under the following process[1].

Transcriptional control: some of the factors are regulating the transcription process. Transcription is the first step for gene expression, in which formation of a gene which is used to form a product, known as a protein. In transcription here the synthesis of RNA molecules from the single stranded DNA.[3] transcription is most important and selective process in the organism [Prokaryotic and Eukaryotic].

Post transcription: it is the biological process, that occur newly transcribed primary RNA [hnRNA], this step occurs prior to the translation of the protein. In capping nacent mRNA involves the addition of the 7 methyl guanosine at the end. In tailing cleavage of the 3 end and then the addition around 250 adenine residues to form a poly A tail also known as a polyadenylation[1].

TRANSLATION

Translation is the universal process in the both eukaryotes and prokaryotes. Translation where protein is synthesis in the cell and it is the second step of genetic expression. Decoding involves in the translation a messenger RNA and using information to build chain of amino acid and polypeptide. In the translation the codons of messenger RNA are read of DNA direction from 5’ to 3’by molecules called transfer RNAs[4]. Translation done in three step: in the process of initiation the ribosome is together with target mRNA. The first tRNA will attached at a start codons. In the elongation, amino acid are brought to the ribosome by the tRNAs and linked together to form a chain. Termination is the last stage of translation, the achieved polypeptide is released to go and do its work on the cell[8].

How is gene expression regulated?

The amount of gene of molecules in a cell describe the function of the cell. The control point for the gene expression is usually at the initiation of transcription. Some of the gene expressed as a part of the cell differentiation process and some are expressed at result of cell differentiation process. Eukaryotes transcription somehow complex the prokaryotic transcription because in the eukaryotic transcription the intervening sequence that are not a part of the mature RNA called introns are removed and are spliced together with the proteins coding region called exons, by the process called splicing.[1]

GENE REGULATION

Transcription, translation and RNA processing (post transcriptional changes) stage is the regulation stage of gene expression. The on–off of transcription process is the main regulatory control of the gene expression in prokaryotes whereas, more complex regulatory mechanism of transcription takes place in eukaryotes.[10]

In Prokaryotes

In cell cytoplasm DNA of prokaryotic organisms floats freely because of singe celled organisms that lack a cell nucleus. Process of transcription and translation occur simultaneously. Transcription stops when resulting protein is no longer needed. Controlling of types and quantity of protein is expressed in a prokaryotic cell is the regulation of DNA transcription. More transcription process occurs when more protein is needed. Therefore control of gene expression at the transcriptional level occur in prokaryotic cells.

In Eukaryotes

There are intracellular organelles in eukaryotic cells which result to complexity. In nucleus DNA is to be founded in eukaryotic cells. Transcription process occurs in nucleus and transcribed into RNA. Newly synthesized RNA transported into cytoplasm, where translation of RNA into protein occur by ribosomes. Transcription and translation process are physically separated by the nuclear membrane, within the nucleus transcription and outside the nucleus in the cytoplasm occur. Regulation of gene expression can occur at: epigenetic, transcriptional, post-transcriptional, translational and post-translationaal[9].

Spatial regulation of Tubulin genes in plant

Tubulin polypeptides is considered as building blocks of microtubules. There are two types of tubulin polypeptides α and β, one molecule of each type form a diamer. These dimmers then assemble in parallel rows to form a hollow cylindrical microtubules. These microtubules aggregets to form complex structure like cilia and flagella. In the cytoplasm and below the plasma membrane around the nuclear membrane. In cell movement these microtubules play important role and responsible for moving chromosomes during mitosis[10].

Temporal regulation of globin genes in animal

In eukaryotes α and β globin is a polyeptides that forms a pocket to bind with oxygen molecules. Gene within each cluster are duplicate of ancestral globin gene, form a small multigene family. During Frame

shifting and chain terminating mutation sometimes abolish the ability to make polypeptide. These noncoding genes are called pseudogenes (ψ).one side cluster genes are expressed in embryo and middle side expressed in fetus and other side genes expressed only after birth. Globin α and β in Arabidopsis expressed in tissue specific manner. Other globin α and β in vertebrates are expressed in temporal pattern during development. .[10]

REGULATOR OF GENE EXPRESSION

Activator

Gene expression activator is a protein that increases the gene transcription of a genes or set of genes. To turn on gene expression, activator is required in eukaryotic cells. To stimulate the assembly and activity of the transcription machinery at gene promoters activators bound to enhancers. To make the control of gene expression easier genes are organized. Immediately upstream of the coding sequence the promoter region is found. Promoter is as longer, the more available space for proteins to bind. This also do more control to the transcription process. The length of the promoter is gene-specific and can differ between genes. The level of control of gene expression can also differ quite dramatically between genes. The promoter is bonded transcription factors that control the initiation of transcription[11].

Just upstream of the transcriptional start site, resides the TATA box Within the promoter region,. This box is a repeat of thymine and adenine dinucleotides. RNA polymerase binds to the transcription initiation complex, allowing transcription to occur. To initiate transcription, a transcription factor (TFIID) is the first to bind to the TATA box. Binding of TFIID recruits other transcription factors, including TFIIB, TFIIE, TFIIF, and TFIIH to the TATA box. RNA polymerase can bind to its upstream sequence Once this transcription initiation complex is assembled. RNA polymerase is phosphorylated ,When bound along with the transcription factors. Result of that of the protein from the DNA to activate the transcription initiation complex and places RNA polymerase in the correct orientation to begin transcription; DNA-bending protein brings the enhancer, which can be quite a distance from the gene, in contact with transcription factors and mediator proteins.

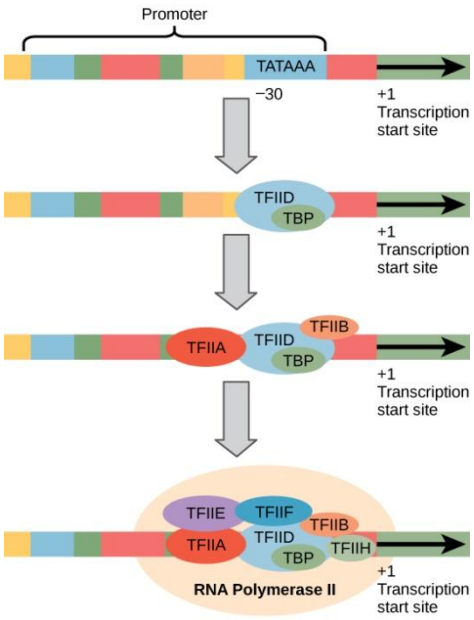

Fig. 1. The process of transcription

There are some regions that help to increase or enhance transcription, which is known as enhancer. Upstream of a gene, within the coding region of the gene,

downstream of a gene, or may be thousands of nucleotides away could be the location of it.

The shape of the DNA changes occur When a DNA-bending protein binds to an enhancer. Interaction between the Activators bound to the enhancers and the transcription factors bound to the promoter region and the RNA polymerase to occur due to this shape changes. DNA is usually depicted as a straight line in two dimensions, which is actually a three-dimensional object. Therefore, a nucleotide sequence thousands of nucleotides away can fold over and interact with a specific promoter[12].

Inhibitors

Inhibitor is the process factor of gene expression in a cell to prevent the expression of a certain gene. Inhibitors is often used in research either transcription or translation process. Ribozymes are catalytic molecules used to inhibit gene expression. By cleaving mRNA molecules, these molecules work. Essentially silencing the genes that produced them. A natural process that cells use to turn down, or silence, the activity of specific genes is RNA interference (rnai). By an enzyme called Dicer the double-stranded molecule is cut into small double-stranded fragments. These small fragments, which include small interfering Rnas (sirna) and microrna (mirna), are approximately 21–23 nucleotides in length. The fragments integrate into a multi-subunit protein called the RNA-induced silencing complex, which contains Argonaut proteins that are essential components of the Rnai pathway.[13]Inhibition of translation step has the effect of blocking protein production and ultimately its function.

METHYLATION

Methylation of DNA: DNA methylation is an epigenetic mechanism that cell use to control gene expression, which occur by the addition of a methyl group [Ch3] to the DNA molecules. Addition of methyl group is controlled at different level of cell and several family of enzyme called DAN methlytransferase [dmts]. Three different dnmts are required for maintenance or establishment of DNA methylation process, [DNMT1, DNMT3a and DNMT3b]. DNMT1 is responsible for the maintenance of the DNA methylation patterns and DNMT3a and DNMT3b is seem to establishment of new de novo DNA methylation patterns. Activity of DNA segment can be change by the methylation without changing the sequence. 5-methylcytosine is found in the human approximately 1.5 percent of genomic DNA. DNA structure can be modified by covalent attachment of methyl group-DNA methylation. In the DNA methylation process is the covalent addition of the methyl group at a 5-carbon of the cytosine base forming in 5-methylcytocine [5-mc],also known as “fifth base” of DNA. DNA methylation is an important component for various cellular process, including embryonic development, X-chromosomes inactivation, genomic imprinting and preservation of chromosomes stability.[13] DNA methylation usually inhibit the transcription of eukaryotes gene and it is particularly abundant in plant and vertebrates. In vertebrates and plant , cpg island [DNA methylation site] occur near many promoter of gene. These cpg island are commonly 10000

to 2000 bp in length and contain high number of cpg site. Methylation of cpg island could enhance and prevent the binding of regulatory transcription factors in the promoter region in another way methylation inhibits transcription is via proteins known as methyl cpg-binding proteins, which methylated sequences[14].

Methylation of histone proteins: in the process of histone methylation, methyl group are transferred to amino acid of histone proteins that makes nucleosomes. In eukaryote cell gene are wrap with the core histone proteins and other chromosomal proteins in the form of chromatin. Methylation of histone proteins may activates and inhibits transcription of genes, because methylation depends on the situation and also depends, which amino acid in the histone are methylated or how many methyl groups are attached. Histone methylation is the covalent modification which commonly occurring on carboxyl group of gulatmate , isoprenylated cysteine and leucine or the side chain of the nitrogen atom of lysine, arginine, and histidine residues. Mass of histone have shown that numerous lysine residues , including lysines 4,9,27 and 36 of H3 lysine 20 of H4 are selected site of methyltion.Histone methylation may be either transcriptional activation and inhibition for example: H3 trimethylation of the histone at a lysine4 [h3k4me3] is active characteristic for transcription, other hand dimethylation of histone H3 at lysine 9 [h3k9me2], is signal for transcription silencing[15].

DEMETHYLATION

Demethylation is the chemical process of removal of methyl group from a molecule. Process of removal of a methyl group from cytosines. DNA demethylation can be passive or active in the absence of methylation of newly synthesized DNA strands by DNMT1 during several replication rounds passive process takes place – for example, upon 5-azacytidine treatment. Direct removal of a methyl group independently of DNA replication is known as Active DNA demethylation. TET dioxygenases, the AICDA deaminase, the MBD4 glycosylase, and the GADD45-alpha BER are an enzymatic complex that involves in active demethylation. GADD45-alpha is induced in response to oxidative stress such as UV light, metal nanoparticles, tobacco smoke, bacterial lipopolysaccharides, and cytokines. DNA demethylation is a first step in reprogramming and is essential for Oct4 transcription (Simonsson and Gurdon, 2004). Failure of demethylation is associated with impaired development in cloned mice embryos[16].

In mammals

In the male pronucleus of zygote immediately after fertilization; In mouse primordial germ cells (pgcs) between E8.5-11.5 day old embryos;[2] Possibly in amphibia - during midblastula transition. Examples of specific DNA demethylation: Genomic imprinting during plant reproduction; Electroconvulsive stimulation-induced demethylation of neurotrophic factor genes in dentate gyrus neurons in the mouse brain. There are several proposed hypothetical mechanisms of active DNA demethylation: A Direct removal of 5-methylcytosine

Direct removal of methyl group

Removal of methylated bases (either by direct removal of methylcytosine, or through cytosine deamination followed by removal of thymine from thymine/guanosine mismatch), followed by insertion of unmethylated one using base excision repair machinery (BER). Removal of entire DNA patch and following filling it with new nucleotides by nucleotide excision repair (NER) or mismatch repair (MMR)[17].

B Removal of 5-methylcytosine via further modified cytosine bases

5-Hydroxymethylcytosine is generated by the Oxidation of the methyl group. TET enzymes can further oxidize 5-hydroxymethylcytosine to 5-Formylcytosine and 5-Carboxylcytosine.

Both the deamination and the oxidation products have been shown to be repaired by TDG, a glycosylase which is involved in base excision repair. A base excision mediated demethylation mechanism would yield double strand breaks if it occurs on large scale in cpg islands.[18] The carboxyl and formyl groups of 5-Formylcytosine and 5-Carboxylcytosine could be enzymatically removed without excision of the base[19].

DNA hydroxymethylation has been proposed to act as a specific epigenetic mark opposing DNA methylation. DNA hydroxymethylation *in vivo* is sometimes associated with labile nucleosomes, which are more easy to disassemble and to be out-competed by transcription factors during cell development. Hydroxymethylation has been associated with pluripotency of stem cells[20].

HISTONE ACETYLATION AND DEACETYLATION

It is the processes in which the lysine residues within the N-terminal tail protruding from the histone core of the nucleosome are acetylated and deacetylated as part of gene regulation. Enzymes with histone acetyltransferase (HAT) or histone deacetylase (HDAC) are responsible for catalysis of reaction. Acetylation is the process where an acetyl functional group is transferred from one molecule to another. Deacetylation is simply the reverse reaction where an acetyl group is removed from a molecule. Acetylation removes the positive charge on the histones, by decreasing the interaction of the N termini of histones with the negatively charged phosphate groups of DNA. As a consequence, the condensed chromatin is transformed into a more relaxed structure that is associated with greater levels of gene transcription. This relaxation can be reversed by HDAC activity. On the NH3+ groups of Lysine amino acid residues the mechanism for acetylation and deacetylation takes place. These residues are located on the tails of histones. The process is aided by factors known as Histone Acetyltransferases (hats). HAT molecules facilitate the transfer of an acetyl group from a molecule of Acetyl Coenzyme-A (Acetyl-coa) to the NH3+ group on Lysine. When a Lysine is deacetylated, factors known as Histone Deacetylases (hdacs) catalyze the removal of the acetyl group with a molecule of H2O. Modification in histone cause dcrese affinity of negative charge binding on DNA due to reduce positive charge on terminal ends[21].

Histone acetyltransferase (hats)

Histone Acetyltransferases, also known as hats, a enzymes that acetylate the histone tails of the nucleosome.

GNAT family

General Control Non-Derepressible 5 (Gcn5) –related N-Acetyltransferases (gnats)- to acetylate substrates Gcn5 is used when it is part of a complex. Recombinant Gcn5 is found to be involved in the acetylation of the H3 histones of the nucleosome.

Histone deacetylase (hdacs)

There are a four classes that categorize Histone Deacetylases (hdacs). Class I includes hdacs 1, 2, 3, and 8. HDAC1 & HDAC2 are in the first class of hdacs. When isolated these enzymes have been found to be inactive which led to the conclusion that they must be incorporated with cofactors in order to activate their deacetylase abilities.DNA binding proteins such as Yin and Yang 1 (YY1) can directly bound to hdacs 1 and 2 . Hdacs 1 and 2 have been found to express regulatory roles in key cell cycle genes including[22].

PHOSPHORYLATION

Protein phosphorylation or phosphorylation, is the attachment of a phosphate group onto a protein. It is done by an enzyme called protein kinases. It uses ATP as a substrate and attach a phosphate group onto a protein – and that protein is phosphorylated. So proteins in the cell that are involved in detecting chemical signals, or proteins that are involved in structural organization and integrity of the cell, proteins that are involved in allowing ions to move back and forth across the membrane, proteins that regulate the expression of genes, are regulated by phosphorylation. When they are phosphorylated, their activity changes and codes a signal that a cell can use in many,different ways.[23] The hydroxyl group containing amino acids of protein namely serine, threonine and tyrosine are subjected to phosphorylation. The role of protein phosphorylation in the control of timing of DNA replication in cell cycle. DNA replication and onset of mitosis, gene expression, nuclear import, development, and memory is regulated by phosphorylation. The cell cycle is mainly regulated by p34cdc2 in association with cyclins B at G2/M and by Cdk2 in association with cyclins A, D1, and E at G1/S checkpoints. MAP kinases might link the G0 to G1 transition with the regulation of the cell cycle whereas phosphorylation of replication protein factors, c-Myc, AP-1, Oct-1, T-antigen, retinoblastoma, and p53 might link the G1 to S transition with the control of DNA synthesis. These transcription regulators can up- or downregulate DNA replication and their DNA binding activities or transacting properties are controlled by phosphorylation.[24] The B subunit of the DNA polymerase (pol) α-primase complex executes an essential role at the initial stage of DNA replication in *Saccharomyces cerevisiae* and is phosphorylated in a cell cycle-dependent manner.

DEPHOSPHORYLATION

Dephosphorylation is the process by which phosphate groups are removed from a molecule by a phosphatase to prevent ligation Removal of phosphate groups from a DNA

fragment[26]. Dephosphorylation employs a type of hydrolytic enzyme, or hydrolase, which cleave ester bonds. Phosphatise is hydrolase subclass used in dephosphorylation. By hydrolysing phosphoric acid monoesters into a phosphate ion and a molecule with a free hydroxyl (-OH) group phosphatase removes phosphate groups. For modifying behavior of a protein the dephosphorylation of proteins mechanism is worked, often by activating or inactivating an enzyme. Components of the protein synthetic apparatus also undergo phosphorylation and dephosphorylation and thus regulate the rates of protein synthesis[25].

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