

AN OVERVIEW ON MECHANISM OF GENE EXPRESSION REGULATION

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Review Article

ABSTRACT

Transcription factors, enhancers, promoters, and translation of genetic code is still in a wide argument of different views in many living organisms for their poorly characterized at the molecular level. It was shown that the coexistence of high mono – methylation levels of lysine 4 of histone H3 is considered a signature of enhancers. Gene expression could be altered by epigenetic and some biotic and abiotic stresses. Some sugars, hormones or amino acids could also alter gene expression. However, some investigators have shown that increased methylation at promoters was associated with down – regulated protein – coding gene expression . In genomic DNA methylation levels, it was found 24 nt small RNAs only were correlated with methylation. Other investigators have set framework for modelling gene regulation, three dimensions were set to draw the graph framework; DNA microstates (vertices), the transitions between microstates, (edges) and the transition rates (edge labels). They believed that their graph will provide a broader foundation for understanding how genes regulate their expressions. More recently, a team of Australian investigators in Medical Res. have shown a new i-motifs; a four – stranded DNA knot in living human cells. These i-motifs were believed to be responsible in helping genes to switch on or off. Future investigations in gene expression will probably be shown in different models than we have learned.

*Key words: transcription factors, enhancers, proteomics, genomics.

الساهوكي وآخرون

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نظرة في آلية تنظيم التعبير الجيني

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باحث

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المستخلص

أن عوامل ومغزات ومحفزات الاستنساخ وترجمة الشفرة الوراثية لازالت كلها في جدل واسع بين الباحثين في مختلف الإحياء، وذلك لعدم وضوح خصوصيتها الجزيئية. لقد تم إيضاح إن مستويات الميثلة الأحادية والثلاثية المرتبطة مع لايسين 4 لهستون H3 تعد دليلاً على فعل المغزات. يمكن للتعبير الجيني ان يغير بفعل بعض عوامل الشد الحية واللاحية، ولكن هذا التغيير لاينتقل للذرية اللاحقة، وكذلك حال بعض الأحماض الامينية والسكريات، ولكن فعل فوق الوراثة يمكن ان يغير التعبير الجيني وينتقل للذرية اللاحقة. اوضح بعض الباحثين ان زيادة الميثلة كانت مرتبطة بفعل تشفير البروتينات. اما في حالة ميثلة DNA الجينوم، فقد وجد ان جزيئات RNAs بطول 24 nt فقط هي المرتبطة اكثر مع الميثلة. وضع باحثون آخرون انموذجاً يتكون من ثلاثة ابعاد لتمثيل تنظيم الفعل الجيني، ووضعوا معادلات خاصة لاعتماده لرسم منحنى الفعل الجيني. مثلت الابعاد الثلاثة بجزيئات DNA، والمسافة الانتقالية بين جزيئات DNA، وسرعة الانتقال. لقد اعتقد هؤلاء الباحثين ان هذا المنحنى يعطي اساساً واسعاً لفهم كيفية تنظيم الجينات لتعبيرها. اكثر حداثة من ذلك، فقد قام فريق من الباحثين الاستراليين في احد مراكز الأبحاث الطبية باكتشاف DNA رباعي الخيط، فيه عقد اطلقوا عليها I.motifs واعتقدوا انها هي المسؤولة عن تفعيل الجين on و off!. إن الأبحاث المستقبلية في هذا الجانب قد تكشف لنا اموراً اخرى حول هذه الاليات لتكون النتائج اكثر وضوحاً وتأكيداً من الفرضيات الحالية لتنظيم الفعل الجيني.

الكلمات المفتاحية: عوامل استنساخ، مغزات، فعاليات بروتينية، جينوم.

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INTRODUCTION

The gene images were different in minds of scientists, due to continuous discoveries in cell organelles functions, miRNAs, and epigenomics. Allard (3) defined genes as: units of inheritance, and they could be in series of alleles. Gardner (16) gave a similar definition but he added that gene is a unit of DNA. However most of living beings; animals and plants have DNA sequences in their chromosomes or cell organelles, but some viruses have RNA nucleotide instead. Genes can be defined as the basic physical – chemical units of nucleotide base pair sequences exist in cell organelles responsible for heredity. So far, only a few cases succeeded in transferring single gene from a genotype into another. Traits of many genes are so difficult to do so for several reasons, such as difficulty in locating the genes, or linkages with other genes of other undesired traits, or due to different gene expression when transferred into another genotype. Genes in general are different in size, in plants and animals. However, do cells regulate gene action or genes regulate their own action is not totally proved, but there are different cases, each of them could show a different mode of action. In this review, we will focus on some articles dealt with these assumptions. Transcription, translation transposons, and epigenetics all could be involved at least in some species of living beings.

REVIEW OF LITERATURE

We have studied years ago that genes should go into transcription via mRNA, and then translated in another organelle, the ribosomes. Some genes do not code for proteins, others do. Bakshi et al. (4) found in italic, that most proteins are translated, while their genes are still being transcribed. This could be due to some barriers, for the DNA replication process (11). This premature translation of some genes has been explained by Adhya and Gottesman (1) as due to transcription termination factor rho proceeds along the nascent RNA to RNA polymerase, that will terminate transcription. Transposons, ribosomes, inhibiting factors, epigenomics, etc all could be involved in mechanisms of transcription and translations of gene DNA. For these reasons, there was no clear evidence of the reality of the mechanism

of gene action to how express their effect of trait or traits in the genotype individual. Fan et al. (16) reported that in prokaryotes, RNA polymerase and ribosomes can bind concurrently to the same RNA transcript. This implies that coupling of translation and transcription will occur at the same time. They also reported that there were direct interactions of RNA polymerase with ribosomes and with large and small isolated subunits of ribosomes. They concluded that, that interaction between RNA polymerase with ribosomes and ribosomal subunits is the reason of coupling transcription and translation. They explained that by assuming that the RNA polymerase binds to one binding site on the ribosomes in the presence of a dimer – monomer equilibrium of RNA polymerase. Another wide scale study on transcription in human B cell precursor acute lymphoblastic leukemia (BCPALL) was carried out by Li et al. (23) on 1223 BCPALL cases, and found different groups of gene expression. This large scale transcriptome sequence analysis of BCP ALL revealed distinct molecular sub – groups that reflect discrete pathways of BCP ALL, informing disease classification and stratification.

eRNA and miRNA in transcription

Probably some of the difficulties in understanding reality of transcription and translation, the existence of some promoters or enhancers. Enhancers are DNA elements contain binding sites for transcription factors involved in transcription of genes or the genetic material of cells. Rahman et al.(32) showed that eRNAs are localized exclusively in the nucleus and are induced with some kinetics as target mRNAs. On the other hand, at the single – allele level, eRNAs are rarely co-expressed with their target loci, demonstrating that active gene transcription does not require the continuous transcription of eRNAs or even their accumulation at enhancers. Meanwhile, they demonstrated that when they co-expressed, sub – diffraction distance measurements between nascent mRNA and eRNA signals reveal that co-transcription of eRNAs and mRNAs rarely occurs within closed enhancer – promoter loops. However, some other researchers (20, 21, 28) found in a genome wide studies in

some cells that eRNA and target mRNA expression was correlated but how eRNAs regulate their target genes was not clear.

mRNA, transcription factors and cell function

The mechanisms of gene regulation or expression are still not clearly defined. However, many researchers have established some techniques in molecular biological systems to reach some of those facts or hypotheses that could explain gene expression at least in the case studied. RNA sequencing and microarray technology are one of helpful tools enabling a wide analyses of transcriptional changes. Crow et al (10) studied three cases in humans; breast cancer subtyping, single cell genomics of pancreatic islet cells, metaanalysis of lung adenocarcinoma, and renal transplant rejection transcriptomics. In all cases, they found hallmarks of generic differential gene expression. They suggested further studies to interpret gene phenotypic associations. Identification of genes that are differentially expressed provides molecular information to answer many questions related to each case studied. In *Arabidopsis thaliana*, Balaguer et al. (5) studied transcription factors in stem cells to understand the initiator and growth of plant tissues and organs. They combined an approach of molecular biology, computational biology and mathematical biology. This approach to some successful findings of factors that could play important roles in stem cell regulation, and in particular quiescent center function. They also concluded that this combinational approach is an efficient one to identify candidate factors in the stem cells. Since, they were able to identify 1625 genes, containing 201 transcription factors which differentially expressed in the stem cells of different growth stages. Payne et al (29) stated that gene regulation is carried out by proteins that bind DNA or RNA molecules at specific sequence. Among those are transcription factors, which bind short DNA sequence to regulate transcription. RNA binding proteins are another group of binding proteins which bind short RNA sequences to regulation RNA maturation, transport, and stability. Cells regulate genes activity in different ways, e.g they regulate transcription through DNA

binding proteins called transcription factors, and they regulate mRNA stability and processing through RNA binding proteins. In their study of these two groups, they found that RNA-mediated regulation is less evolvable than transcriptional regulation for that mutations are less likely to create interactions of an RNA molecule with a new RNA binding protein than they are to create interactions of gene regulatory region with a new transcription factor. However, they did not explain the reason of that difference affinity, but they believed that their finding suggests a reason why a specific kind of gene regulation is especially abundant in living beings.

miRNAs and histone methylation

Through the last two decades, many researches have been done on small non-coding RNA (miRNAs), especially those of 20-24 nt. Guo et al. (19) stated that these small molecules function as diverse endogenous gene regulators at the post-transcriptional level. They developed a knowledge-based database called it Plant miRNA Encyclopedia (PmiRNA), which based on uniform processing of sequenced small RNA libraries using miRDeep-P₂, followed by manual curation using newly updated miRNA identification criteria, and comprehensive annotation. PmiRNA when used contained 16,422 high confidence novel miRNA loci in 88 plant species and 3,966 retrieved from miRBase. However, they concluded that PmiRNA is hierarchically accessible and has eight built-in search engines. So, they believe that PmiRNA is useful for plant miRNA cataloguing and data mining. On the other hand, Elsahookie (12,14) reported that gene regulation or expression could be altered by some severe abiotic factors, such as high salinity stress, tissue culture media, severe drought and even some sugars and amino acids, but these changes are not expected to be transmitted to next generations.

Epigenomics:

Epigenetics play a role in gene expression in many cases of different genera and species of living organisms. Epigenetics takes place in histone and DNA of cells, that will change the phenotype of the individual traits. O'Green et al. (26) investigated the activity of a broad

collection of genomically targeted epigenetic regulators that could write epigenetic marks associated with a repressed chromatin state. They found that d Cas 9 fusions produced target gene repression over a range of zero to 10 folds that varied by locus and cell type. Whereas, they found that d Cpf1 fusions were unable to repress gene expression. The most persistent gene repression required the action of several effector domains, however, KRAB-d Cas 9 did not contribute to persistence in contrast to previous reports. Surprisingly, the gene repression was not correlated with deposition of either H₃ K₉ me₃ or H₃ K₂₇ me₃. According to their results, they suggested that the so-called repressive histone modifications are not sufficient for gene repression. It was previously found that epigenetic modification control cellular gene expression profiles and maintain the differentiation state of cells (1,7,23). More recently Stepper et al. (34) studied DNA methylation using some chimeric methyltransferase to understand their relationship to transcription. This targeted epigenome editing is a new technique in molecular biology. They have employed a DNA methyltransferase Dnmt3a Dnmt3 – L programmable targeting domain to introduce DNA methylation into the human genome specifically at the Ep CAM, CXCR4, and TFRC gene promoters. Their results showed that targeting of these loci with single gRNAs leads to efficient and widespread methylation of the promoters. Multiplexing of several guide RNAs does not increase the efficiency of methylation. The targeted methylation peaks were observed around 25 bp upstream and 40 bp downstream of the PAM site, while 20 -30 bp of the binding site italic se were protected against methylation. They concluded that potent methylation is dependent on Dnmt3a / Dnmt 3L complexes on the DNA. On the other hand, they have found that the introduced methylation causes transcriptional repression of the targeted genes.

Binding elements and mutagenic activity:

Methods or techniques used to determine location of transcription factor binding sites are useful for better understanding of gene regulation in all living beings. Human is the most important genus among animal kingdom

to understand processes of gene regulation, transcription, translation, and methylation. This will help researchers to cure some of difficult disorders in human body. Chen and Sadowski (8) have developed a technique called it serial analysis of binding elements. This involves subtractive hybridization of chromatin precipitation – enriched DNA fragments followed by the generation and analysis of concatamerized sequence tags. They searched for p 53 target genes in the human genome, and were able to identify several p 53 targets in addition to numerous potentially novel targets, including the DNA mismatch repair gene MLH1 and PMS2. They also found that these two genes were responsive to DNA damage and p53 activation in normal human fibroblast, and have p53-response elements within their first intron. They believed that these two genes may serve as a sensor in DNA repair mechanisms, and a critical determinant for the decision between cell cycle arrest and apoptosis. More recently, Perayasamy et al. (30) studied the cytosine deaminase activity of apolipoprotein BmRNA editing enzyme catalytic polypeptide – like (APOBEC) genes as an important source of mutation in diverse cancers. The most important result of their work suggests that as p53 is frequently mutated in cancer, and the loss of p53 promote cancer mutagenesis.

Chromatin proteomics and Transcription factors

Over the last decade, transcriptome analysis revealed that only 1-2 % of the genome serve as a template for protein biogenesis, whereas up to 80 % of the genome is transcribed (31). The vast majority of the human genome is pervasively transcribed into non-coding RNAs. Long non-coding RNAs (lncRNAs) are defined as transcripts of more than 200 nucleotides without evident protein coding functionality (6). Chen et al (9) reported that metastasis – associated lung adenocarcinoma transcript 1 (MALAT1) is a broadly expressed lncRNA involved in many aspects of cellular processes, they have employed a high – throughput strategy to characterize the interacting proteins of MALAT1 by combining RNA pull down, quantitative proteomics, bioinformatics, and experimental validation, in their approach, they were able

to identify 127 potential MALAT1-interacting proteins and established a highly connected MALAT1 interactome network consisting of 788 connections. Meanwhile, they showed that MALAT1 was highly involved in five biological processes: RNA processing, gene transcription, ribosomal proteins, protein degradation, and metabolism regulation. They also found that MALAT1 binding competes with the interaction between sirtuin1 and depleted breast cancer 1 (DBC1), which then releases sirtuin 1 and enhances its deacetylation activity. Then, the deacetylation of p53 reduces the transcription of a spectrum of its downstream target genes, promotes cell proliferation and inhibits cell apoptosis. These results just uncovered a novel mechanism by which MALAT1 regulates the activity of p53 through the lncRNA-protein interaction. Enhancers play an important role in setting cell- and time-specific gene expression programs. They are able to promote transcription of their target gene at a considerable distance, up to several hundred kb (27). Enhancers are characterized by DNA hypersensitivity to nucleases, nucleosome-depleted regions and clustering of DNA binding-motifs for transcription factors (18). Soldi et al. (33) stated that despite enhancers have a key role in modulating transcription, but they are still poorly characterized at the molecular level, and their limited DNA sequence conservation in evolution and variable distance from target genes make their unbiased identification challenging. The coexistence of high mono-methylation and low tri-methylation levels of lysine 4 of histone H3 is considered a signature of enhancers. However, they added that a comprehensive view of histone modifications associated with enhancers is still lacking. For this reason, by combining chromatin immunoprecipitation (ChIP) with mass spectrometry, they were able to investigate cis-regulatory regions in macrophages to comprehensively identify histone marks specifically associated with enhancers, and to profile their dynamics after transcriptional activation elicited by an inflammatory stimulation. Their results revealed the existence of novel subpopulations of enhancers, marked by specific histone modification signatures. However, the

combinational code of histone modifications, highlighted the potential of proteomics in addressing fundamental questions in epigenetics.

DNA methylation and genomic imprinting

Gene transcription and translation has been studied via many investigators on different cells of eukaryotes and prokaryotes. One of crop plants which is important and widely studied is maize (*Zea mays* L.) Liu et al (25) used immature embryos of an inbred line of maize (18-599R), cultured on an optimized medium, then graded embryos into three stages. That was to study methylated DNA, gene expression, small RNA-sequence and beyond. They obtained very nice detailed data on their own research targets. They have observed that most of the differentiated embryos exhibited a methylation increase compared to normal embryos. Increased methylation at promoters was associated with down-regulated protein-coding gene expression, but the correlation was not strong. Callus and immature embryos analysis indicated that the methylation increase was induced during induction of embryonic callus, suggesting phenotypic consequences may be caused by perturbations in genomic DNA methylation levels. The only significant correlation between small RNAs and DNA methylations was found with 24nt RNAs. They suggested extending epigenetic changes during maize embryo callus formation, and the methylation changes might explain some of the observed embryonic callus variation in callus formation. Elsahookie (13) stated that gene expression in such a case could be transmitted to next generations. Genomic imprinting is an epigenetic phenomenon that allows monoallelic expression of a subset of genes dependent on parental origin which regulated by DNA methylation (15, 22). However, this phenomenon is not necessarily to be alone in gene regulation, besides transcription, translation, promoters, enhancers, ... etc. So, gene regulation used to be analysed with an assumption that its mechanisms of regulation was in general in equilibrium. This could hold true in analyzing binding and unbinding of transcription factors. Ahsendorf et al. (2) have studied a new approach in modelling gene regulation. They

have introduced a graph –based framework that can accommodate non- equilibrium mechanisms. A gene–regulatory system was described in a graph ,which specifies the DNA microstates (vertices) , the transitions between microstates (edges) and the transition rates (edge labels). The graph yields a stochastic master equation for how microstates probabilities change over time. They also showed that this framework has broad scope by providing new insights into three very different models. Then, they have concluded that as epigenomic data become increasingly available, we can represent gene function with graph, as gene structure has been represented by sequences, and that the method they introduced will provide a broader foundation for understanding how genes work. Finally, and more recently, Zeraati et al. (35) showed in their article that i- motifs (four – stranded knot of DNA) has been found in living human cells. They believed that these: knots = i- motifs, are responsible in helping genes to switch on or off. This new finding of DNA knots will change a lot of theories and models on gene regulation, transcription, translation, and other related enhancers and genomics and epigenomics in human and other living beings.

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