Gene expression regulation

How do mammalian cells regulate and control gene expression?

Abstract.

Gene expression regulation is a complicated process that is controlled at many different levels. Not surprisingly, there are many factors that influence gene expression both molecular and environmental. The central dogma of biology provides a clear platform for the study of gene regulation and it involves a) Initiation b)Elongation and c)Translation. Initiation of transcription is probably the most important step in gene regulation and and certainly the best understood. In this study we have described studies that have looked at different aspects of gene regulation such as chromatin structure and promoters.

Background.

The different cell types in multicellular organisms arise due to the synthesis and accumulation of different sets or RNA and protein molecules. Comparison of the detailed banding patterns that are detactable in condensed chromosomes at mitosis are evidence that large blocks of DNA are not lost during development in mammalian organisms. (Alberts et al, 2008)

The central dogma of biology

Chromatin structure and epigenic control of gene regulation

In eukaryotic cells, DNA is contained in the nucleous and packed in chromosomes. Chromosomes are very long molecules of DNA which are wrapped around histone proteins to form nucleosomes. The nucleosome is the major subunit of chromatin which does not just serve a structural role but plays a critical role in transcriptional regulation. Chromatin can repress

gene expression by inhibiting access of the transcriptional factors to the DNA. (Nestler et al, 2002)

Activation of gene expression requires that cells alleviate nucleosome mediated repression of an appropriate subset of genes. This is achieved by activator proteins that modify chromatin structure. The activation process, involving transcription factors, along with histones and cofactors, displaces or remodels chromatin, and opens up regions of the DNA and the core promoters of genes, for the binding of regulatory proteins to the core promoter, and the recruitment of RNA polymerase. This process is called transcription initiation. (Nestler et al, 2002)

Each nucleosome is composed of an octamer of core histones (fig. 1) (two each of H2A, H2B, H3 and H4), around which two super-helical turns (80 bp) of DNA are wrapped. Given that DNA is compacted into a highly condensed and ordered structure, considerable interest has focused on how the transcriptional machinery gains access to the genes contained within chromatin and expresses them in an organized program, as is required in the processes of cellular differentiation and development. The change of chromatin organization via its covalent modificationis thought to provide access to the genes for the transcription apparatus. The post-translational modifications of the histone tails which include reversible acetylation, phosphorylation and methylation, play a critical role in the dynamic condensation and relaxation that occurs during the cell cycle. (Ito, 2007) This is refered to as epigenic control.

Epigenetics is the regulation of gene expression involving chromatin-based pathways, and includes 3 distinct, but highly interrelated, mechanisms: DNA methylation, histone density and posttranslational modifications, and RNA-based mechanisms. (Matouk, 2008)

Dna Methylation

Both DNA methylation and chromatin modification are believed to regulate gene expression. Many studies have attempted to provide insights into the mechanism of transcriptional silencing by a methyl-CpG binding protein MeCp2. MeCP2 is also shown to interact with Sin3/Histone deacetylase co-repressor complex. It has also been shown that inhibition of histone deacetylases by specific inhibitors could reactivate endogenous genes previously scilenced by DNA methylation. (Ng, 1999)

Histone modification

DNA methylation gene silencing is closely linked to histones deacetylation. Methylation of histones at specific lysine residues, works in concert with acetylation and other histone modifications to provide a code that could determine heritable transcriptional states. (Fahrner et al, 2002)

It has been reported that acetylated histone H3 and methyl-H3-K45 are enriched in

euchromatic domains and positively correlate with active gene expression. Methyl-H3-K9, on the other hand, is enriched at heterochromatic regions. It has been widely suggested that these histone modifications, determine the status of active versus inactive gene expression. (Fahrner et al, 2002)

TATA binding Proteins

Te binding of TPB to the TATA box is usually the first step in the assembly of the preinitiation complex at the promoter. The recruitment of the transcription machinery is a rate limiting step in gene expression. Many of the activators and repressors known exert their effects through interactions with TBP or with proteins bound to TBP. TBP also interacts with other basal transcription factors such as TFIIA, -IIB, and -IIF, with RNA polymerase II and many other components of the transcriptional machinery (Kim et al, 1997)

One such other component are the zink fingers which are DNA-binding motifs consisting of specific spacings of cysteine and histidine residues that allow the protein to bind zinc atoms. The metal atom coordinates the sequences around the cysteine and histidine residues into a finger-like domainwhich can interdigitate into the major groove of the DNA helix.

Promoter elements in TATA – less promoters.

The best-characterized core promoter element so far, is the TATA box. It is recognized by the TATA-binding protein subunit of TFIID and initiates PIC formation. However, TATA box is not present in all gene promoters. Therefore it is necessary for alternative mechanisms to be employed in order to start PIC formation. The downstream promoter element (DPE) is an important component of TATA-less promoters as it is recognized by components of TFIID other than TBP. The initiator element (Inr) spans the region where mRNA synthesis begins and nucleates PIC formation via TFIID components. It can either function independently or in combination with a TATA element and exerts a positive effect on transcription. (Deng et al, 2005)

Inhibition of gene expression by short double stranded RNA.

Short interfering RNAs (siRNAs) are double-stranded RNAs of around 21-25 nucleotides. They have been known to function as key intermediaries in triggering RNA interference in invertebrates. siRNAs have a characteristic structure, with 5*-phosphatey3*-hydroxyl ends and a 2-base 3* overhang on each strand of the duplex. (Caplen et al, 2009)

Report on Current Literature.

How Does RNA Polymerase Transcribe Through a Histone-Covered Template?

The question arises as to how the " activated" chromatin is transcribed? Felensfeld et al (1996) attempted to find and answer to this question.

They conducted experiments in which a single nucleosome core particle was ligated into a plasmid so that it lay between an SP6 polymerase promoter upstream and transcription terminators downstream. The template was transcribed, and the position of the octamer was determined. (Felensfeld et al 1996)

The results of this study showed that a histone octamer is not an insuperable obstacle to the passage of an RNA polymerase and that the octamer can be transferred around the enzyme without releasing its grip on DNA. . On longer templates, the octamer can travel to any vacant site on the DNA and this is probably governed by the probability of " ring closure. " The use of short DNA segments, however, limits the final positions available to the octamer, (Felensfeld et al 1996).

Histone modification

Fahner et al (2002) used SW480 cells to examine the relationship between DNA hypermethylation and specific histone code components at a silenced tumor suppressor gene promoter in human cancer. The cells were initially treated with 1 μM 5-Aza-dC (Sigma) for periods of 12, 24, 48 h, or 5 days or else with 300 nM TSA (Wako) for 24 h. They were then maintained in McCoy's 5A modified medium. RKO cells were maintained in MEM. All media (Invitrogen) were supplemented with 10% fetal bovine serum and 1%. They used ChIP, PCR and reverse transcriptase PCR as well as MSP to conclude that DNA methylation may directly, or indirectly inhibit transcription and maintain key elements of the histone code at hypermethylated gene promoters in cancer.

DNA methylation

The effect of DNA methylation on the transcriptional activity of the hamster adenine phosphoribosyltransferase (aprt) and the herpes thymidine kinase (tk) genes has been investigated by Keshet et al, (1985). M13 constructs were used containing these gene sequences. Specific segments of each gene were methylated in vitro by restriction fragment primer-directed second-strand synthesis using the substrate 2′-deoxy-5-methylcytidine triphosphate (dmCTP). These hybrid-methylated molecules were then inserted into mouse Ltk- cells by DNA-mediated

cotransfer.

In all cases, the integrated sequences retained the in vitro-directed methylation pattern. The aprt gene was inhibited by CpG methylation in the 5′ region but was unaffected by methylation at the 3′ end or in adjacent M13

sequences. In contrast to this, DNA methylation in both the 5′ promoter region and the 3′ structural region of the tk gene had a strong inhibitory effect suggesting that this modification may affect transcription by a mechanisms that does not involve the direct alteration of recognition sequences for RNA polymerase. (Keshet et al, 1985)

Zink fingers

The fusing the TATA box-binding protein (TBP) to other DNA-binding domains, such as zink fingers may provide a powerful way of targeting TBP to particular promoters. To explore this possibility, (Kim et al, 1997) constructed a fusion protein, TBPyZF. In this fusion protein the three zinc fingers of Zif268 were linked to the COOH terminus of yeast TBP. Gel shift experiments revealed that this fusion protein formed an extraordinarily stable complex when bound to the appropriate composite DNA. In vitro transcription experiments and transient cotransfection assays revealed that TBPyZF could act as a site-specific repressor. Because the DNA-binding specificities of zinc finger domains can be systematically altered by phage display, it may be possible to target such TBPyzinc finger fusions to desired promoters and thus specifically regulate expression of endogenous genes. (Kim et al, 1997)

A DNA fragment encoding TBP/ZF was generated by PCR and cloned in pET11a (Novagen). yTBP and TBP/ZF were produced as fusion proteins with His Tag and purified by metal chelation affinity chromatography (Novagen). Gel shift assay was performed and the sample was analysed by gel electrophoresis followed by In Vitro Transcription Analysis. (Kim et al, 1997)

Transient Cotransfection Assay was used to make Reporter plasmids by cloning five GAL4 binding sites and a promoter sequence in pGL3-Basic. The 293 cells were transfected by calcium phosphate precipitation with a glycerol shock and then luciferase activity was measured using luciferase assay reagent (Promega) and a ML2250 Luminometer (Dynatech). (Kim et al, 1997)

Promoter elements in TATA-less promoters.

(Deng et al, 2005) have defined a core promoter element located downstream of the TATA box and is recognized by TFIIB DNA-binding domain. This core promoter is distinct from the helix-turn-helix motif. (Deng et al, 2005)

The group used G9E4 spanning nucleotides −51 to +12 from the Adenovirus E4 promoter and the following expression vectors pEThTFIIB, pETHIS- hTBP, pETGal4AH. Recombinant TFIIB (and derivatives) were purified and TFIIB promoter derivatives were generated using Quickchange site directed mutagenesis. Recombinant hTBP was purified with Nickel agarose. The AdE4 DNA template used contained seven nucleotides downstream of the TATA box and PCR primers used were RSP1 and RSP2, with restriction sites that are recognized by BamHI and EcoRI. (Deng et al, 2005)

The products of primer extension were initially purified on 6% native polyacrylamide gel and then the radiolabeled DNA fragments were used to perform a bandshift assay together with recombinant hTFIIB and hTBP. The band containing complexes of TBP-TFIIB was excised after detection with autoradiography, then DNA fragments were recovered by incubating the gel

slice overnight and precipitation with ethanol. The recovered DNA fragments then were used as a PCR template for the next cycle of selection.

At the end of a seven round selection, the DNA fragments were digested with BamHI and EcoRI, which was then followed by cloning into pGEM3. A series of assays were then performed including, methylation interference analysis, transcription assays, Hirt assay, and TFIIB depletion of nuclear extracts. (Deng et al, 2005)

Inhibition of gene expression by short double stranded RNA.

Caplen et al (2001) investigated whether siRNAs can be used directly to inhibit gene expression by first assessing interference in C. elegans by using siRNAs corresponding to C. elegans unc-22. They generated dsRNA molecules by mixing sense and antisense ssRNA oligomers. The integrity and the dsRNA character of the annealed RNAs were confirmed by gel electrophoresis. All mammalian cells used were grown in DMEM primary mouse embryonic fibroblasts (MEFs) from wild-type I129 mouse embryos and were expanded to generate a more homogenous cell line. They then carried out Cell Culture and Nucleic Acid Transfections using standard methods. Analysis of Gene Expression was carried out using a series of techniques. GFP expression was assessed in mammalian cells by fluorescence-activated cell sorter. To assess cell survival Caplen et al (2001) carried out In Vitro Kinase Assays and labeled proteins were visualized by autoradiography of dried gels.

Discussion.

Inhibition of gene expression by short double stranded RNA.

Caplen et al, (2009) found in her study that synthetic siRNAs can induce gene-specific inhibition of expression in Caenorhabditis elegans and in cell lines from humans and mice. As well as that, they found that the interference by siRNAs was superior to the inhibition of gene expression mediated by single-stranded antisense oligonucleotides. The siRNAs seem to avoid the nonspecific effects triggered by longer double-stranded RNAs in mammalian cells. From the results of this study it emerges that t Although mammals and lower organisms seem to share dsRNA-triggered responses that involve a related intermediate (siRNAs), it is likely that there will be differences and similarities in the underlying mechanism. Their study, however, does not address any possible differeces in these mechanisms. These observations may open a path towards the use of siRNAs as a reverse genetic and therapeutic tool. (Caplen et al, 2009).

Zink fingers

Kim et al, (1997) showed, by using Gel shift experiments, that the fusion protein TBP/ZF formed a very stable complex when bound to the appropriate composite DNA site. In vitro transcription experiments and transient cotransfection assays, carried out by the group, revealed that TBP/ZF could act as a site-specific repressor. The DNA-binding specificities of zinc finger domains can be systematically altered by phage display. Given that recent results suggest that it could be possible to select zinc finger proteins that will specifically recognize almost any desired site(Greisman et al, 1997), it may be, therefore, possible to target such TBP/zinc finger fusions to desired promoters in order to specifically regulate expression of endogenous genes. (Kim et al, 1997)

Promoter elements in TATA-less promoters.

Deng et al, (2005) have defined a core promoter element which is located downstream of the TATA box and is recognized by TFIIB. As it was mentioned earlier, this is a DNA-binding domain that is distinct from the helix-turn-helix motif and regulates transcription in a manner that is promoter context-dependent. They also found that TFIIB HTH motif, which has contact the BREu, is not conserved in all eukaryotes. However, the TFIIB recognition loop which contains G153 and R154 is indeed conserved in all eukaryotes. They showed that TFIIB recognizes two sequence elements that flank the TATA box, employing in this way, independent DNA-binding motifs and exerting its effect in the transcription regulation. (Deng et al, 2005)

The experiments using the aprt gene by Keshet et al, (1985) showed that the 5′ region of the gene is influenced by methylation, while the 3′ region is not. As well as that, their results indicate that methylation may play a regulatory role over the entire gene domain.

Regarding the role of chromatin structure on gene expression, questions are now being addressed as to how transcriptionally active chromatin is generated, how it is maintained and how its limits are defined. It is also neccessary to understand how a polymerase that binds to a promoter interacts with its chromatin environment, during initiation and chain elongation. (Felsenfeld et al, 1996).

Fahrner et al, (2002) suggested that DNA methylation, by inhibiting transcription, may directly or even indirectly maintain key elements of the

histone code at hypermethylated gene promoters in cancer. (Fahrner et al, 2002)

Conclusions.

The regulation of gene expression has huge implications on many fields of science. It is a process that exerts it effects and is influenced by many factors. One of the very promising fields where gene expression could make an impact is cancer research and adjuvant therapy.

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