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Deoxyribonucleic Acid (DNA), Ribonucleic Acid (RNA) and Protein Biosynthesis and the Regulation of Their Interactions in Cloning

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ABSTRACT

The biosynthesis of DNA, RNA and protein are essential processes which cells carry out in different ways. The regulation of these processes varies from cell to cell but ultimately enhances the production of desired products as well as gives a better understanding to the functionality of the genes. The process that cells and viruses use to regulate the way the information in their genes is turned into gene products varies. Although a functional gene product may be an RNA or a protein, the majority of known mechanisms regulate protein coding genes. Any step of the gene's expression may be modulated, from DNA-RNA transcription to the post-translational modification of a protein. In cloning the regulation of the synthesis of DNA, RNA and protein occurs in the individual process resulting in the outcome of a 'perfectly' cloned gene. Furthermore, gene regulation drives the processes of cellular differentiation and morphogenesis, leading to the creation of different cell types in multicellular organisms. This review takes a look at some of the ways in which these processes are regulated.

DNA, RNA and Protein Biosynthesis

DNA synthesis also referred to as DNA replication is the process in which an exact copy of parental DNA is made with the parental molecule serving as a template. The expression of the information encoded in the base sequence of DNA begins with the synthesis of an RNA copy of the DNA sequence making up a gene. RNA synthesis which is also known as transcription is the process in which single – stranded RNA with a base sequence complementary to the template strand of DNA is synthesized. Translation, also known as protein synthesis is the process by which the genetic message carried by messenger RNA (mRNA) directs the synthesis of polypeptides with the aid of ribosomes and other cell constituents [1].

In 1953, Watson and Crick suggested how DNA might be replicated and since then various researches have gone forth to discover more about the DNA. Replication patterns differ in prokaryotes and eukaryotes. In prokaryotes for e.g. *Escherichia coli* synthesis occurs at the replication fork, the place in which DNA helix is unwound and individual strands are replicated, at the completion of the replication, a structure like the Greek alphabet theta is formed. RNA synthesis generates three 3 kinds of RNA –messenger RNA (mRNA) which bears the message for protein synthesis, transfer RNA (tRNA) which carries amino acid during protein synthesis and ribosomal RNA (rRNA) which are components of ribosomes [1].

Regulation and Interaction

Willis and Rhind (2009) [2] discovered that cells showed slow replication in response to DNA damage. This slowing was the first DNA damage checkpoint response discovered and its study led to the discovery of the central checkpoint kinase, Ataxia Telangiectasia Mutated (ATM). The checkpoint could slow bulk replication by inhibiting replication origin firing or slowing replication fork progression. The S-phase DNA damage checkpoint reduced but did not absolutely halt DNA synthesis in the presence of damaged DNA during S-phase. The hallmark of the S-phase DNA damage checkpoint was the slowing of replication in response to DNA damage. In addition to checkpoint-dependent slowing, bulky DNA lesions could also slow replication forks independently of checkpoint activity. Thus, the contribution of fork slowing to the overall reduction in DNA synthesis is dependent on the density of DNA damage [2].

According to Minocha *et al*., (1991) [3] various inhibitors of polyamine biosynthesis were used to study the role of polyamines in DNA synthesis and cell division in suspension cultures of *Catharanthus roseus* (L). DL α-difluoromethylarginine inhibited ADC activity, cellular putrescine content, DNA synthesis, and cell division. Methylglyoxal bis (guanylhydrazone) inhibited S-adenosylmethionine decarboxylase (EC 4.1.1.50) activity without affecting DNA synthesis and cell division.

DNA replication blockage in various differentiated cells was investigated on the model of heterokaryons. Two distinct types of DNA synthesis regulation in heterokaryons "differentiated cell and proliferating cell" were revealed: I. Neutrophils and nucleated erythrocytes efficiently prevented the entry of non-malignant proliferating cells nuclei into the S-period but usually failed to substantially inhibit the replication in malignant cells nuclei. Both mortal and immortalized proliferating cells activated the DNA synthesis in neutrophil and chicken erythrocyte nuclei. II. Macrophages did not influence the DNA synthesis in the nuclei of non-malignant cells in heterokaryons but drastically inhibited that in the nuclei of malignant cells. Only immortalized cells reactivated DNA synthesis in the nuclei of macrophages [4]. DNA synthesis regulation in heterokaryons between mouse neutrophils and cultured cells of various proliferative potentials has also been studied. The following features were found. Both immortalized and non-immortalized cells can reactivate DNA synthesis in neutrophil nuclei. Neutrophils inhibit the entry of cultured cell nuclei into S phase and have no effect on ongoing DNA synthesis. Malignant cells are much less sensitive to the inhibitory action of neutrophils than non-malignant ones. Non-malignant immortalized cells are as sensitive to this effect as non-immortalized cells. Neutrophil karyoplasts do not influence DNA synthesis in partner cultured cell nuclei [5].

Mouse erythroleukemia cells were treated with the topoisomerase II poison VP-16, the intrastrand crosslinking agent cis-DDP, and the ribonucleotide reductase inhibitor hydroxyurea. In all cases, the rate of DNA synthesis decreased as a result of the treatment. The rate of DNA synthesis in the reactions containing nuclei isolated from untreated cells and extracts from cells treated with the three drugs were slightly reduced and did not show significant differences between the drugs. In the systems containing nuclei from cells treated with cis-DDP, DNA synthesis was again slightly inhibited; synthesis in nuclei treated with hydroxyurea was enhanced, and synthesis in the systems containing nuclei from cells treated with VP-16 was significantly reduced. DNA synthesis was reduced to the same extent in a system containing nuclei isolated from untreated cells that had been briefly sonicated to introduce a limited number of double-strand breaks in the DNA. From the foregoing it means, there is a topologic mechanism for regulation of DNA synthesis in the S phase of the cell cycle [6].

The most studied member of the serine proteinase inhibitor superfamily, ovine uterine serpin (OvUS), inhibits proliferation of several cell types. It was tested whether inhibition of DNA synthesis in human prostate cancer (PC-3) cells involved cytotoxic actions of OvUS or the induction of apoptosis. Recombinant OvUS blocked proliferation of PC-3 cells at concentrations as low as 8 μg/ml as determined by measurements of [^{3}H] thymidine incorporation or ATP content per well. Results from flow cytometry experiments showed that OvUS blocked the entry of PC-3 cells into S phase and the exit from G_2/M phase. The results also indicated that OvUS acts to block cell proliferation through disruption of the cell cycle dynamics rather than induction of cytotoxicity or apoptosis. The finding that OvUS can regulate cell proliferation makes this one of only a few serpins that functions to inhibit cell growth [7].

The effect of intrinsic curvature upstream of a bacterial promoter on the efficiency of transcription was first reported in the early 1980s. To date, there are countless examples indicating the importance of a curved DNA sequence during steps of transcription, mainly in regulating the transcription initiation process. It has been recently shown that global transcription factors as well as several other transcriptional regulators have a significant tendency to regulate operons with curved DNA sequences in their upstream regulatory regions (8). Transcription of stable RNAs and mRNA genes were analyzed. In the presence of guanosine-3', 5'-(bis)pyrophospahte ppGpp a slight general enhancement of specific pauses in all transcription systems was noted. Pausing enhancement requires the presence of ppGpp during elongation but not during initiation. The results obtained underline the importance of pausing for transcription regulation and offer a plausible explanation for inhibition of stable RNA expression under conditions of elevated concentrations of ppGpp [9].

Although in bacterial cells all genes are transcribed by RNA polymerase, there are 2 additional enzymes capable of catalyzing RNA synthesis: poly (A) polymerase I, which adds poly (A) residues to transcripts, and primase, which produces primers for DNA replication. Mechanisms of actions of these 3 RNA-synthesizing enzymes were investigated for many years, and schemes of their regulations have been proposed and generally accepted. Nevertheless, recent discoveries indicated that apart from well-understood mechanisms, there are additional regulatory processes, beyond the established schemes, which allow bacterial cells to respond to changing environmental and physiological conditions. These newly discovered mechanisms include: (i) specific regulation of gene expression by RNA polyadenylation, (ii) control of DNA replication by interactions of the starvation alarmones, guanosine pentaphosphate and guanosine tetraphosphate, (p)ppGpp, with DnaG primase, (iii) a role for the DksA protein in ppGpp-mediated regulation of transcription, (iv) allosteric modulation of the RNA polymerase catalytic reaction by specific inhibitors of transcription, rifamycins, (v) stimulation of transcription initiation by proteins binding downstream of the promoter sequences, and (vi) promoter-dependent control of transcription antitermination efficiency [10].

Kurland and Maaløe (1962) [11] discovered that chloramphenicol (CM) initially accelerates the synthesis of transfer as well as ribosomal RNA when cells are growing in minimal medium. This effect according to them is absent in an amino acid medium. High concentrations of CM, which inhibit most protein synthesis, relieve the need for an *external* supply of a required amino acid for RNA synthesis. However, a lag precedes the resumption of RNA synthesis when CM is added to amino-acid-starved cells. These results suggest that the rate of RNA synthesis is determined by the internal amino acid concentration. This may be accomplished by a repressor mechanism in which the transfer RNA acts as repressor and amino acid adenylate as inducer. At high CM concentration, the rate of RNA synthesis decreases progressively with time. At intermediate CM concentrations the amount of protein synthesized is paralleled by an *increase* in RNA synthesis, over and above the fixed amount produced at CM concentrations which completely block protein synthesis. Under these conditions the synthesis of ribosomal RNA decreases steadily whereas transfer RNA is synthesized at a constant rate [11].

The global regulatory nucleotides (p) ppGpp are major effectors for the control of ribosomal RNA in bacteria. Inhibition occurs at various steps during initiation but also during elongation where RNA polymerase pausing is observed. The biosynthesis of

ribosomes is determined, however, by the rate of rRNA synthesis while the synthesis of the protein components is a subordinate process. In fact, the synthesis of rRNAs is controlled by a complex set of interacting regulatory networks. In summary, the hormone-like effector molecules (p)ppGpp are able to trigger two types of transcriptional regulation, stringent and growth rate control. Both types of regulation are linked to changes in the cellular concentration of the effector nucleotides, which are either in the micro- or the millimolar range [12].

Regulation of translation occurs primarily in the initiation phase. Secondary structures at the mRNA ribosomal binding site (RBS) inhibit translation initiation. The accessibility of the RBS is regulated by temperature and binding of small metabolites, proteins, or antisense RNAs. Translation initiation is promoted by initiator factor IF1, IF2, and IF3, which mediate base pairing of the initiator tRNA anticodon to the mRNA initiation codon located in the ribosomal P-site [13]. Insulin rapidly activates protein synthesis by activating components of the translational machinery including eIFs (eukaryotic initiation factors) and eEFs (eukaryotic elongation factors). In the long term, insulin also increases the cellular content of ribosomes to augment the capacity for protein synthesis. The rapid activation of protein synthesis by insulin is mediated primarily through phosphoinositide 3-kinase. Inhibition of mTOR by rapamycin markedly impairs insulin-activated protein synthesis. mTOR controls translation initiation and elongation. Insulin induces dephosphorylation and activation of eEF2 to accelerate elongation. Insulin also stimulates synthesis of ribosomal proteins by promoting recruitment of their mRNAs into polyribosomes [14].

Gene expression can be regulated at the level of initiation of protein biosynthesis via structural elements present at the 5' untranslated region of mRNAs. These folded mRNA segments may bind to the ribosome, thus blocking translation until the mRNA unfolds. In the stalled state, the folded mRNA prevents the start codon from reaching the peptidyl-tRNA (P) site inside the ribosome. Upon repressor release, the mRNA unfolds and moves into the mRNA channel allowing translation initiation. A comparative structure and sequence analysis suggests the existence of a universal stand-by site on the ribosome (the 30S platform) dedicated for binding regulatory 5' mRNA elements. Different types of mRNA structures may be accommodated during translation preinitiation and regulate gene expression by transiently stalling the ribosome [15]. Maintenance of cell homeostasis and regulation of cell proliferation depend importantly on regulating the process of protein synthesis. Many disease states arise when disregulation of protein synthesis occurs. Most translational controls occur during the initiation phase of protein synthesis, with the initiation factors being the major target of regulation through their phosphorylation. However, translation, especially of specific mRNAs, may also be regulated by sequestration into processing bodies or stress granules, by *trans*-acting proteins or by microRNAs. When the process of protein synthesis is hyper-activated, weak mRNAs are translated relatively more efficiently, leading to an imbalance of cellular proteins that promotes cell proliferation and malignant transformation. This occurs, for example, when the cap-binding protein, eIF4E, is over expressed, or when the methionyl-$tRNA_i$-binding factor, eIF2, is too active. The importance of the translation initiation factors as regulators of protein synthesis and cell proliferation makes them potential therapeutic targets for the treatment of cancer [16]. The activation of human resting T lymphocytes results in an immediate increase in protein synthesis. The increase in protein synthesis after 16–24 hours has been linked to the increased protein levels of translation initiation factors. The regulation of protein synthesis after 1 hour of activation was studied using αCD3 antibody to stimulate the T cell receptor and αCD28 antibody to provide the co-stimulus. Activation of the T cells with both antibodies led to a sustained increase in the rate of protein synthesis. The initial increase in protein synthesis was accompanied by activation of the guanine nucleotide exchange factor, eukaryotic initiation factor (eIF) 2B, and of p70 S6 kinase and by dephosphorylation of eukaryotic elongation factor (eEF) 2. A new finding was that the p38 MAPK α/β pathway was involved in the regulation of overall protein synthesis in primary T cells. Both eIF2B and p70 S6 kinase play important roles in the regulation of protein synthesis during the early onset of T cell activation [17]. The future challenge is to obtain atomic-resolution structures of complete initiation complexes in order to understand the mechanism of translation initiation in molecular detail [13].

The 18 kDa histone H1-like protein from *Chlamydia trachomatis* (Hc1) is a DNA-binding protein thought to be involved in condensation of the chlamydial chromosome during late stages in the chlamydial life cycle. Expression of Hc1 in *E. coli* results in an overall relaxation of DNA and severely affects DNA, RNA and protein synthesis. The interaction of Hc1 with single-stranded DNA and RNA by Southwestern and Northwestern blotting was analysed. It was further revealed that purified, recombinant Hc1 dramatically affects transcription and translation in vitro at physiologically relevant concentrations. These results were found to coincide with the formation of condensed Hc1-DNA and Hc1-RNA complexes as revealed by agarose gel electrophoresis and electron microscopy [18]. Previous studies have shown various platinum containing compounds to be effective anti-tumor agents in man and animals. Many of these compounds have also been shown to be effective inhibitors of bacterial DNA, RNA and protein synthesis. Some of these new derivatives appear to be nearly 3-fold more potent than the more thoroughly studied *cis*-diamminedichloroplatinum(II) (*cis*-PDD) and *trans*-diamminedichloroplatinum(II) (*trans*-PDD) [19].

CONCLUSION

The DNA, RNA and protein synthesis are very important processes in cell cycles. The individual synthesis and regulation of each of these processes is vital in cloning and gene expression as it helps to give a better understanding to the functioning of the genes and cells. Although cells have ways of regulating these processes, external factors are sometimes employed to yield the appropriate outcome of the genes and products desired. More research is however needed to further discover ways of regulating these processes to bring out hidden potentials within the genes.

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