Difference between prokaryotic and eukaryotic transcription

SUMMARY

Biosynthesis of proteins is under direct control of DNA in most cases or else under the control of genetic RNA where DNA is absent. Information for structure of a polypeptide is stored in a polynucleotide chain (Gupta, 2007). Sequences of bases in a particular segment of a polypeptide chain will determine the sequence of amino acids in a particular polypeptide (Gupta, 2007). The relationship popularly known as central dogma explains how protein synthesis is controlled by nucleic acids. There are two major steps involved in protein synthesis (1) transcription and (2) Translation. Transcription involves transfer of genetic information from DNA to mRNA and Translation involves translation of language of nucleic acids in to that of proteins (Gupta, 2007). Transcription will be discussed in detail in the present topic.

TEXT

Transcription is the synthesis of RNA which carries the genetic information present in DNA (Fig. 1). The DNA is double stranded and can theoretically code for two separate RNA molecules (Jain, 2000). However, it has been found that only one of the two strands of the gene is transcribed (Jain, 2000). Only in a few exceptional cases both strands are transcribed. This is possible because the promoter is asymmetrical and unidirectional (Jain, 2000). The DNA strand which have a sequence homology with the RNA is known as the coding strand. The second strand which is complementary to RNA and serves as the template for RNA synthesis is known as the non-coding strand. It is therefore, a misnomenclature, as it is the non-coding strand which is in fact transcribed to form the primary transcript (Jain, 2000).

Transcription in prokaryotes

The principal enzyme involved in transcription is the DNA dependent RNA polymerase (commonly called as RNA pol). To understand the transcription, it is necessary to learn more about the RNA polymerase. The bacterial RNA polymerase consists of five polypeptide chains including two chains of α (alpha) polypeptide and one chain each of β (beta) and βÊ¹ (beta dash) and σ (sigma) polypeptides (Fig.). The RNA polymerase molecule thus can be represented as α2ββÊ¹σ, in which the attachment of sigma (σ) factor is not very firm, so that the core enzyme (α2ββÊ¹) can be easily isolated. The size and function of the prokaryotic RNA polymerase is given in table 1.

The active sites of core enzyme are shown in fig. (). Once RNA synthesis is initiated, σ dissociates after RNA is 8-9 bases long and then the core enzyme brings about elongation of mRNA. The dissociated sigma factor may again combine with core enzyme to form RNA polymerase holoenzyme (Fig,..).

Events in transcription

The entire process of transcription can be divided in to following steps:

(1) Template recognition

(2) Initiation

(3) Elongation and

(4) Termination

Template recognition

The promoter directs the RNA polymerase to recognize the correct region of the gene and to bind at this site. The -35 region serves this function and is recognized by the enzyme. The size of the RNA polymerase is such that about 60 nucleotides in the gene are involved in binding of the enzyme to the template. Sigma factor plays an important role in specific binding of the enzyme with the template (Jain, 2000). The core enzyme without the sigma factor can bind to DNA but the binding is not promoter specific. The sigma factor is thus necessary for the formation of promoter-enzyme complex. The binding of RNA polymerase to a site other than the promoter is generally referred as loose binding. In presence of sigma factor, the affinity for loose binding is reduced while the affinity for specific binding is increased (Jain, 2000). Thus the chances of only the specific binding taking place are enhanced many fold in presence of sigma factor (Jain, 2000).

Initiation and elongation of RNA synthesis in prokaryotes

RNA synthesis by RNA polymerase proceeds in four steps: (i) the holoenzyme first binds at the promoter site, forming the closed promoter complex in which DNA remains double helicle, (ii) the closed complex isomerizes and causes unwinding and separation of DNA strands to form open (binary) promoter complex, (iii) after unwinding only one of the two strands is copied; this is achieved by incorporation of nucleotides, initially without movement of enzyme leading to the formation of RNA chain, up to 9 bases in length. During the incorporation of these 9 bases, at every step, there is a possibility for the release of this small RNA chain, a process described as ‘ abortive initiation’ (Gupta, 2007). A cycle of abortive initiation usually occurs

generating a series of short (2-9 base) oligonucleotides, before initiation is usually successful. (iv) Once initiation succeeds, the sigma factor of RNA polymerase dissociates. (v) the dissociation of sigma factor marks the entry of NusA protein, which helps elongation, and promotes pausing and termination at specific sites. Core enzyme now undergoes a major conformational rearrangement and a stable ternary elongation complex is formed. This complex moves along DNA, synthesizing RNA all along its path at a rate of about 40 bases per second at 370C (Gupta, 2007). Elongation of RNA transcript continues till an unstable termination complex is formed (Gupta, 2007).

Termination

The termination of mRNA chain in prokaryotes is brought about by certain termination signals on DNA. These DNA sequences providing termination signals are called terminators (Gupta, 2007). Once the enzyme hits the terminator, it falls off the template and the transcription stops. The termination signals whenever found on DNA can be of two types: (i) Rho (ρ) dependent termination and (ii) Rho (ρ) independent termination.

(i) Rho (ρ) dependent termination:

The termination factor (ρ) participates in this type of termination (Jain, 2000). Rho (ρ) is a 46 KD protein and its active form is a hexamer, having a total mass of 275 KD. It binds to growing RNA chain and moves along the RNA. Once rho catches the RNA polymerase, it results in chain termination. The question arises how?. It has been found that once RNA polymerase hits the terminator sequences, it pauses for a short time. During this period the

rho factor reaches the RNA polymerase and causes it to fall off the template. Once RNA polymerase is detached, the RNA chain also comes off and the transcription terminates (Fig...).

(i) Rho (ρ) independent termination:

In some genes, there is a definite region of intrinsic sequences which causes the termination of RNA chain. This includes two G: C rich stretches at the end of RNA transcript which are complementary to each other. These form a 7-20 bp intra-molecular hairpin structure. Further this region is followed by a small stretch of U residues which form relatively weak interaction with dA residues of the gene (Fig..). such a structure is highly unstable thermodynamically and causes the displacement of newly synthesized RNA from the DNA template. Once the RNA is detached, the RNA polymerase falls off and the termination of transcription occurs. This type of termination provides an interesting example where the structure of RNA itself can cause its own termination from the DNA chain.

Transcription in Eukaryotes

The eukaryotes have more than one type of RNA polymerase. Based on the activity to α-aminitin, an antibiotic which inhibits mRNA synthesis, three classes of RNA polymerases have been identified which are involved in the transcription of different class of eukaryotic genes. There properties are given in table 2.

The eukaryotic RNA polymerases are large molecules of Ëƒ500KD in size. It has two large subunits of -200KD and -140KD, respectively. The 200 KD subunit is similar to βÊ¹ subunit of E. coli of RNA polymerase and have

similar function (the template binding). Besides these two proteins it also has upto 10 different small subunits. A subunit of Pol II, which has similarity with one of the subunits present in Pol I and also in Pol III, is similar to the α-subunit of E. coli enzyme and helps in the enzyme assembly. Besides the RNA polymerase, a number of other transcription factors are also needed for the transcription.

Promoter sites for Initiation of transcription

Promoters for RNA polymerase I could not be initially studied since all genes for rRNA were similar. Promoters for RNA polymerase III, on the other hand, had some unusual downstream promoters. However for RNA polymerase II, several hundred eukaryotic genes have now been sequenced and their promoters studied revealing some general features in three regions located at start point, centred at sited lying between -25 bp and -100 bp. The least effective of these three regions is the TATA or Hogness box (7 bp long) located 20bp upstream to the start point.

The TATA box is surrounded by G-C rich sequences and is comparable to pribnow box of prokaryotes. Further upstream is another sequence called CAAT box, which being necessary for initiation, is conserved in some promoters (β globin gene), but is not necessary in some other genes. This sequence lies between -70 and -80 bp. Another sequence called GC box (GGGC GG) is found in one or more copies at -60 or -100 bp upstream in any orientation in several genes. It has been shown that CAAT and GC boxes determine the efficiency of transcription, while TATA box aligns RNA polymerase at proper site, with the help of TFIID and other transcription factors (Gupta, 2007).

Initiation

In eukaryotes the initiation is more complex. It involves a number of specific transcription factors. The process has been followed for the Pol II action resulting in the synthesis of mRNA. The process is essentially similar for Pol I and Pol III. For initiation, it requires, a number of trans acting factors along with the RNA polymerase. The trans acting factors, which are the product of various regulatory genes, bind to either DNA or to each other or to RNA polymerase. They can also bind in various combinations. All the transcription factors involved with Pol II are called TF II. First the factor TF IID binds to TATA box (-15 to -21 region) covering about 25 nucleotides within the -17 to -42 region. Now factor TF IIA associates itself to the complex, further extending the protected region towards upstream, upto the -55 to -80 region. On the other hand TF IIB associates itself protecting the region at -10 to +10. It binds to two strands in a non-symmetrical manner. This complex prepares the stage for binding of RNA polymerase II which covers up to +15 region on template strand and 5 extra nucleotides (up to +20) on the non-template strand. Finally TF IIE joins, extending the protection upto +30 region. Once the entire complex has been assembled, the incorporation of first nucleotide takes place.

Transcription factors and elongation of RNA chains in eukaryotes

Certain accessory proteins of transcription, called the elongation factors enhance the overall activity of RNA polymerase II, leading to increase in elongation rate. Atleast two such proteins (transcription factors) are known (i) the transcription factor TFIIF accelerates RNA chain growth relatively

uniformly, in concert with RNA polymerase II or pol II, (ii) transcription factor TFIIS (also called SII) helps elongation of RNA chain, by relieving the obstructions in the path of such elongation. TFIIS is known to function by first causing hydrolytic cleavage at 3Ê¹ end of RNA chain, which are stuck and can not elongate. Thus RNA polymerase moves backwards (hydrolytic cleavage) under the direction of TFIIS before it moves forwards through the block to elongation (fig.) (Gupta, 2007).

Termination of RNA synthesis in eukaryotes

In eukaryotes, the actual termination of RNA polymerase II activity during termination may take place through termination sites similar to those found in prokaryotes (the nature of individual termination sites is not known). But these termination sites are believed to be present away (sometimes up to one kilobase away) from the site of 3Ê¹ end of mRNA. Obviously 3Ê¹ end of mRNA will be generated due to post-transcriptional cleavage. This cleavage, at the end, is believed to be achieved by what is popularly called ‘ snurp’ (small nucleur RNA-protein complex). Snurp used for post-transcription cleavage has not been identified so far but is believed to be certainly different than the U1 snurp, which is believed to be involved in intron splicing in split genes. Moreover, a sequence 5Ê¹ AAUAAA 3Ê¹ has been found just on the 5Ê¹ side of poly(A) addition site in several eukaryotic mRNAs. The poly(A) tail is added to 3Ê¹ end of eukaryotic mRNA after processing of precursor mRNA. The sequence 5Ê¹ AAUAAA 3Ê¹ in mRNA 3Ê¹ end seems to be common in eukaryotic mRNA and mutation in this sequence cause elongation of mRNA. This will suggest that this sequence contains the signal for endonucleolytic post-transcriptional cleavage. This sequence

therefore, is not involved in the termination of the synthesis of mRNA, but helps in generating 3Ê¹ end later through endonuclease cleavage, in which snurp helps in an unknown manner.

FAQs

Q. What is transcription?

Ans: synthesis of RNA which carries the genetic information present in DNA.

Q. What is the composition of RNA polymerase in prokaryotes?

Ans: RNA polymerase consists of five polypeptide chains including two chains of α (alpha) polypeptide and one chain each of β (beta) and βÊ¹ (beta dash) and σ (sigma) polypeptides.

Q. What is the function of sigma factor of RNA polymerase in prokaryotes?

Ans: The function of sigma factor in prokaryotes is Promoter recognition and initiation of transcription.

Q. What are the steps in transcription?

Ans: The entire process of transcription can be divided in to following steps: (1) Template recognition, (2) Initiation, (3) Elongation and (4) Termination.

Q. What is a Promoter?

Ans: Promoter is defined as a sequence of DNA having the signal which directs the proper binding of RNA polymerase to DNA and activates it to a form which is capable of initiating the transcription.

Q. What is the role of NusA protein?

Ans: NusA protein, helps in elongation, and promotes pausing and termination at specific sites in prokaryotic transcription.

Q. How termination of transcription occurs in prokaryotes?

Ans: The termination of mRNA chain in prokaryotes is brought about by certain termination signals on DNA. These DNA sequences providing termination signals are called terminators (Gupta, 2007). Once the enzyme hits the terminator, it falls off the template and the transcription stops. The termination signals whenever found on DNA can be of two types: (i) Rho (ρ) dependent termination and (ii) Rho (ρ) independent termination.

- Q. How many RNA polymerases are involved in eukaryotic transcription?

Ans: three classes of RNA polymerases (Pol I, Pol II and Pol III) have been identified which are involved in the transcription of different class of eukaryotic genes.

Q. What the functions of Pol I, Pol II and Pol III?

Ans: The functions of Pol I is Ribosomal RNA synthesis, Pol II is mRNA synthesis and Pol III is tRNA synthesis, 5S and other small RNA synthesis.

Q. What are transcription factors?

Ans: transcription factors are proteins which are needed for initiation of transcription, but are not a part of RNA polymerase.

Q. What is Hogness box?

Ans: The second region of eukaryotic promoter which is similar to -10 region of prokaryotes. is called the ‘ TATA box’ or ‘ Hogness box’.

Q. What is the role of transcription factor TFIIF and TFIIS?

Ans: the transcription factor TFIIF accelerates RNA chain growth relatively uniformly, in concert with RNA polymerase II or pol II while transcription factor TFIIS helps elongation of RNA chain, by relieving the obstructions in the path of such elongation.

Q. How TFIIS helps in elongation of RNA?

Ans: TFIIS is known to function by first causing hydrolytic cleavage at 3Ê¹ end of RNA chain, which are stuck and can not elongate.

Q. what is the role of small nucleur RNA-protein complex?

Ans: Termination takes place at termination sites which are present away from the site of 3Ê¹ end of mRNA. The 3Ê¹ end of mRNA will be generated due to post-transcriptional cleavage. This cleavage, at the end, is believed to be achieved by ‘ snurp’ (small nucleur RNA-protein complex).

Q. How does Rho (ρ) helps in termination of transcription?

Ans: When RNA polymerase hits the terminator sequences, it pauses for a short time. During this period the rho factor reaches the RNA polymerase and causes it to fall off the template.

MCQs:

1. Transfer of genetic information from DNA to mRNA?

a. translation b. transcription

c. transformation d. All of the above

2. During transcription the DNA strand which have a sequence homology with the RNA is known as:

a. coding strand b. non-coding strand

c. Both a and b d. None of the above

3. During transcription the strand which is complementary to RNA and serves as the template for RNA synthesis is known as?

a. coding strand b. non-coding strand

c. Both a and b d. None of the above

4. The principal enzyme involved in transcription is:

a. RNA polymerase b. DNA polymerase

c. transcription factor d. a and b only

5. RNA polymerase is:

a. RNA dependent b. DNA dependent

c. protein dependent d. hormone dependent

6. The RNA polymerase molecule thus can be represented as:

a. αβ2βÊ¹σ b. αββÊ¹σ2

c. α2ββÊ¹σ d. αββÊ¹2σ

7. The function of α subunit is:

a. Template binding b. Nucleotide binding

c. Both a and b d. Enzyme assembly

8. Sequence of DNA having the signal which directs the proper binding of RNA polymerase to DNA is known as:

a. Hogness box b. promoter

c. CAAT box d. None of the above

9. The sigma factor is necessary for the formation of:

a. promoter-enzyme complex b. Enzyme assembly

c. CAAT box d. All of the above

10. The dissociation of sigma factor marks the entry of NusA protein:

a. TF IIB b. TF IIE

c. TF IIS d. Nus A protein

11. Termination of transcription in prokaryotes is:

a. Rho (ρ) dependent b. Rho (ρ) independent

c. both a and b d. a only

12. Hairpin structure for termination of transcription is found in:

a. Rho (ρ) dependent b. Rho (ρ) independent

c. both a and b d. a only

13. which RNA polymerase is found in Eukaryotes:

a. Pol I b. Pol II

c. Pol III d. All of the above

14. TATA box of eukaryotes is comparable to which sequence of prokaryotes:

a. pribnow box b. CAAT box

c. Hogness box d. All of the above

15. Transcription factors helps in:

a. initiation b. elongation

c. termination d. a and b only

16. At what region of DNA does RNA polymerase first bind to a gene:

a. Initiation site b. Transcribed region

c. Promoter d. Intron

17. RNA polymerase adds new nucleotides to the growing RNA’s at what end?

a. 3Ê¹ end b. 5Ê¹ end

c. both a and b d. none of the above

Key: 1-b, 2-a, 3-b, 4-a, 5-b, 6-c, 7-d, 8-b, 9-a, 10-d, 11-c, 12-b,

13-d, 14-a, 15-d, 16-c, 17-a.

ASSIGNMENTS/TUTORIALS

Q. 1: Difference between prokaryotic and eukaryotic transcription initiation.

Q. 2: Explain the role of RNA polymerase in prokaryotes.

Q. 3: Eukaryotes contain multiple RNA polymerases explain their role?

Q. 4: Explain Rho dependent and Rho independent termination of transcription in prokaryotes.

Q. 5: What are transcription factors? discuss the role.