Molecular Genetics

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I. Mechanism of Transcription Initiation and Elongation

I.1. DNA Dependent Synthesis of RNA

DNA dependent RNA polymerases require: a. RNTPs b. Mg^{2+} c. Zn^{2+}

It acts in the 5′ - 3′ direction. It requires DNA for its activity and is most active when it is bound to double stranded DNA.

Features:

- Does not require primers to initiate. *Initiation occurs when the polymerase binds to the promoter sequences in DNA.*
- The 5′ triphosphate group oif the newly formed RNA strand remains intact throughout the transcription process.
- During the elongation phase of transcription, the newly formed RNA strand forms a temporary double stranded helix with the DNA, and then peels off shortly after formation.

Promoter Melting

Transcription initiation requires formation of the open promoter complex (RPo). To generate RPo, RNA polymerase unwinds the DNA duplex to form the transcription bubble and loads the DNA into the RNAP active site.

DNA rotation in transcription bubble:

Transcription takes place in a short unwound region of the DNA helix called the **transcription bubble**. Movement of this bubble down the helix requires significant rotation of the helix, that is prevented by DNA binding proteins. This problem is solved by RNA polymerase, as it generates waves of positive supercoils ahead of the transcription bubble and negative supercoils behind it. It is assisted by topoisomerases.

A few definitions:

1. Promoters *direct the transcription of adjacent sequences by binding of RNA polymerase to it.*
2. *A protein binding site, represented by a* consensus sequence, *may be a short sequence of nucleotides which is found several times in the genome and is thought to play the same role in its different locations. For example, many transcription factors recognize particular patterns in the promoters of the genes they regulate.*

THe DNA strand used as the template for RNA synthesis depends on gene to gene. But we know, that promoter sequences are asymmteric, and a gene typically has only a single promoter. This restricts the RNA pol to bind only in a specific direction, and thus in the process the choice of the template strand for the RNA synthesis is also specified.

Binding and Initiation

Transcription initiation is generally divided into two phases, binding and initiation.

Binding Phase
RNA polymerase, guided by a bound σ subunit, binds to DNA at a promoter sequence.

Mechanism:
The formation of a closed complex at the promoter begins when the TATA binding protein binds to the TATA box and is bound by TFIIB. This is next bound by TFIIF which, in turn, guides the RNA pol II to the promoter. FInally two other subunits bind to create the **closed complex**.

Promoter melting, which is essentially the creation of an open complex from a closed complex, is due to the helicase activity of a subunit called TFIIH, that promotes the unwinding of DNA near the RNA start site, with the help of ATP hydrolysis.

Initiation Phase
The initiation phase encompasses transcription initiayion and promoter clearance. Promoter clearance often involves breaking contact with transcription factors involved only in the initiation phase and making contacts with elongation specific factors.

Abortive Initiation

The complex will usually go through something known as abortive initiation which is caused by the polymerase repeatedly initiating transcription and that will cause the release of small RNA strands. This will usually occur until the polymerase can form a longer RNA strand and it will move past the abortive initiation stage. The complex will then stop 25-30 bp before the promoter sequence which will allow the complex to make a transition to allow for promoter escape.

Mechanism of Initial Transcription

1. During the initial stages of transcription, the RNA polymerase remains bound to the promoter, unwinds downstream DNA amd pulls it onto itself.
2. The unwinding of the DNA does not require energy from ATP hydrolysis, instead, it enables the RNA pol, and the DNA to be more stable and hence is feasible.
3. It is believed that this process provides the energy required by polymerase to break the polymerase–promoter and core–s interactions associated with escape. Thus, scrunching is a way to store and mobilize energy during transcription initiation, and its release upon escape is what enables polymerase to break free of the promoter and dislodge s factor from the core.

Elongation, termination and release

Elongation factors, like elongin, greatly increase the activity of the RNA pol II during the elongation phase. They ensure in increasing the probability of the enzyme not deattaching from the RNA before it reaches the end of the gene. These factors supress pausing between the transcription process and also coordinate protein compexes involved in the post transcriptional modification of the mRNA. To ensure that the RNA pol traverses smoothly through the chromatin structures in eukaryotes, the enzyme is also associated with chromatin remodelling complexes that structurally remoles the chromatin for easy traversion.

I.2. Promoter-RNA pol Interaction

The precise sequence of the promoter region, encode how strong its nteraction with RNA pol is. Promoters that encode for abundant protiens are strong promoters, while promoters that code for rare proteins, are weak promoters.

EMSA

A mobility shift assay is electrophoretic separation of a protein–DNA or protein–RNA mixture on a polyacrylamide or agarose gel for a short period . The speed at which different molecules (and combinations thereof) move through the gel is determined by their size and charge, and to a lesser extent, their shape. The control lane (DNA probe without protein present) will contain a single band corresponding to the unbound DNA or RNA fragment. However, assuming that the protein is capable of binding to the fragment, the lane with a protein that binds present will contain another band that represents the larger, less mobile complex of nucleic acid probe bound to protein which is ‹ shifted › up on the gel (since it has moved more slowly).

However, this test is only qualitative, and does not provide us with quantitative data.

DNA Footprinting Assay

Footprinting identifies DNA sequences binding to a particular protein.

1. DNA fragment thought to contain sequences that bind to a RNA binding protein.
2. They then add the RNA binding protein to it.
3. Random breaks are induced in the strand using DNAse after the tip of one strand is radioactively labeled.
4. The part where the RNA polymerase binds is not acted upon bu the DNAase.
5. Fragments are separated by gel electrophoresis, and only labelled bands are seen.
6. The missing part reveals the sequences that the protein binds.

The important advantage here is the accuracy with which we can identify the binding sequences, without affecting the binding affinity of the protein in the process.

I.3. Termination

Intrinsic terminators (ρ-independent)

RNA polymerase pauses at a variety of sequences, some of which are terminators. One of the two outcomes is then possible, the polymerase either bypasses the site and continues, or it undergoes a conformational change that disrupts the RNA-DNA hybrid. An A=U hybrid at the 3′ end of the transcript is relatively unstable, and the RNA polymerase dissociates completely. This is the usual outcome at terminators.

ρ dependent Termination

ρ dependent terminators lack the A residuesin the template strand, but usually include a CA rich sequence called the *rut* element.The ρ binds to the RNA sequence at specific binding sites. It then moves forward in the 5′–3′ direction until it reaches the transcription complex that is paused at a termination site. Here it contributes to the release of the RNA transcript by virtue of its RNA-DNA helicase activity.

I.4. RNA processing

In eukaryotic cells RNA processing occurs in the nucleus, while in prokaryotes, it occurs in the cytoplasm.

Capping, splicing and tailing are the three components of RNA processing.

Capping of RNA

Three enzymes molecules bind to the phosphorylated tail of the RNA pol II molecule, lying in wait for the 5′ end of the RNA to emerge. These enzymes are

1. phospohatase
2. guanyl transferase
3. methyltransferase

As soon as the new 5′ RNA emerges, the last nucletide is converted to a monophosphate by the phosphatase, to which a GMP is added in reverse linkage (5′–5′). After that a methyl transferase adds methyl group to the gunyl residue, creating 7-methylguanosine cap. This cap helps the cell to distinguish between the mRNA and the other kinds of RNA molecules inside the cell.

Splicing of RNA molecules

Splicing is the ricess of removal of introns that do not code for protein from the RNA transcript to make mature mRNA. For this, premRNA undergoes two subsequential phosphoryl-transfer events that are termed as transesterification; these join two exons while removing the intron as a lariat. The 5′ bond is broken before the 3′ bond, and a new phosphodiester bond is formed fter the intron is removed.

Why does the cell invest in splicing if it is so wasteful a process?
Ans: Splicing, by virtue of generating many intron-exon complexes, actually give room for many new fuctional proteins to appear. The proteins we observe today have been proves to be essentially a patchwork of several functional protein subunits called domains, that are independent. THe existence of introns of various lengths actually allow for these different domains to recombine and give rise to new protiens.

Since the number of phosphodiester bond remains the same, this process could theoretically occur without any NTP synthesis. However the process of splicing is extremely complex, and about 50 prpoteins, in the form of a spliceosome, carry out the procedure.

Spliceosomes
Interestingly, RNA splicing is performed largely by RNAs instead of proteins. RNA molecules recognise intron-exon borders and participate in splicing. These special RNAs, known as snRNAs, are each associated with protein subunits to form snRNPs. These snRNPs form the core of a spliceosome. The spoliceosome uses ATP hydrolysis to produce a complex series of RNA-RNA rearrangements.

Plasticity in RNA splicing

Interestingly, the process of RNA splicing shows remarkable plasticity. The choice of splice sites depend on the affinity of the 5′ & 3′ junctions and the branch point of the RNA for the splicing machinery.