

3/4/02

1. Name the TBP-containing factors involved in Pol I, Pol II, and Pol III transcription, respectively. (6 pts.)

SL1, TFIID, TFIIB

2. During eukaryotic Pol II transcription, which TF complex binds to the RNA polymerase away from the promoter? (3 pts.)

TFIIF

3. A gel-shift assay indicates that SL1 and TFIIB cannot shift the Pol I and Pol III promoter regions as purified proteins. Explain. (6 pts.)

They both need assembly factors to be recruited to the promoter region (UBF for SL1 and TFIIC for TFIIB).

4. What causes the eukaryotic Pol II to turn into an elongation complex? Which TFII complex performs this function? (5 pts.)

CTD phosphorylation by TFIIF

5. Name at least two TFII factors that functionally resemble prokaryotic sigma factor and explain how they are similar to sigma. (6 pts.)

Any two of the following

TFIID—contains TBP which can recognize the TATA-box sequence

TFIIB—involved in bringing RNA Polymerase to the promoter region

TFIIF—associates with RNA polymerase away from the promoter region

TFIIF—modifies RNA polymerase so that it turns into an elongation complex

6. Pol III transcription involves an internal promoter. Describe how the transcription start site is determined with the internal promoter. (4 pts.)

TFIIC will bind to the internal promoter and recruit TFIIB onto the 5' of the transcription initiation site. TFIIB will recruit the PolIII complex near the transcription initiation site so that transcription start at the +1 position.

Therefore, the internal promoter is used only to position TFIIB and Pol III at the right position.

7. The cis-regulatory sequences for 3' end processing include AAUAAA sequence and G/U-rich sequence at the 3' of the primary transcript. Are these sequences found in the mature mRNA? Explain. (5pts.)

AAUAAA sequence is present in the mature mRNA since RNA is cleaved at the 3' to this sequence for polyadenylation.

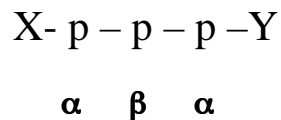
Primary mRNA is cleaved at the 5' of the G/U-rich sequence. This 3' portion of the nascent transcript is rapidly degraded (since it's not capped nor adenylated). Thus G/U-rich sequence is not on the mature mRNA.

8. One can find 5'-5' phospho-linkage in mature mRNA transcripts.

a) Where is this linkage found and how is it generated? (6 pts)

5' Cap--a GMP molecule is connected to the first residue using 5'->5' phospholinkage.

b) Label the phosphates found on this linkage (α , β , or γ). (3 pts.)



9. Briefly describe the following assay and state when one would use it. (6 pts.)

a. DNase Hypersensitivity assay

Purified nuclei with intact chromatin are subjected to a mild DNase treatment. DNA is then extracted and digested with restriction enzymes. DNase-sensitive regions are revealed as indicated by the cuts on the DNA generated only upon DNase treatment. This method is used to determine the open chromatin regions where transcription factors are likely to bind.

b. S1 Nuclease assay

Antisense single strand DNA probe is generated and hybridized to the mRNA. The hybrid is then subjected to S1 nuclease to determine which part of the DNA is represented on the mRNA. This method is used to determine the 5' end, 3' end or exon-intron junctions.

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Bonus

What are the experimental advantages and limitations of using the in vitro system to study eukaryotic transcription? (3 pts.)

Any of the following;

advantages:

-the roles of individual factors can be studied by omitting/adding them to reactions. For example the role of TFIID and TFIIE in promoter escape or the role of TAFs in activation.

-transcription can be easily divided into multiple steps (preinitiation complex formation, promoter escape, elongation)

-promoter sequences can be manipulated to study the effect you are interested in

limitations:

-results might not apply to an in vivo system. for example many experiments used TBP instead of TFIID. this is not the case in the cell.

-chromatin templates are not used

-the effects of proteins and factors not present in the system cannot be studied