11.4 Transcription initiation by Pol II (p.469-471)

1. sequential binding of general txn factors
   • position pol
   • melt DNA

Fig 11-27

Assembly of Pol II Pre-initiation complex

TFIID = TBP + TAF's

TFIIH
• helicase
• kinase
• DNA repair

Assembly of Pol II Pre-initiation complex
1. the specific sequences required for txn factor binding?

2. the order of txn factor assembly?

3. the protein domains important for DNA binding or txn factor activity?

How would you experimentally determine:

11.5 Molecular Mechanisms of Txn Activation and Repression (p. 469-481)

1. general functions of activators and repressors

2. heterochromatin

3. deacetylases

4. acetylases

5. chromatin-remodeling

6. mediators, DNA bending

7. cooperativity

Activators & Repressors:

1. modulate chromatin structure

2. interact with Pol II and TF’s

Chromatin-mediated repression

1. “Silencer”

2. Proteins (RAP1) recognize silencer

3. Other proteins recruited (SIR’s)

4. Histone binding, deacetylation

5. Condensation, spreading
Repressors can direct histone deacetylation
1. DNA binding domain
2. repressor domain
3. recruitment of deacetylases

(a) Repressor-directed histone deacetylation
corepressors

Activators can direct histone hyperacetylation
1. DNA binding domain
2. activator domain
3. recruitment of acetylases

(b) Activator-directed histone hyperacetylation
co-activators

The “Histone Code”
Modifications affect interactions with:
1. DNA
2. proteins

Euchromatin (active/open)
H3 ARTKQ7ARTKSTGGKAPRKL
H3 ARTKQ7ARTKSTGGKAPRKL
H4 SGRGKGGKGLGGKAAKRHK
H4 SGRGKGGKGLGGKAAKRHK

Heterochromatin (inactive/condensed)
H3 ARTKQ7ARTKSTGGKAPRKL
H3 ARTKQ7ARTKSTGGKAPRKL
H4 SGRGKGGKGLGGKAAKRHK
H4 SGRGKGGKGLGGKAAKRHK

The chromatin state of specific genes - “ChIP”
Cross-linked chromatin
(chemicals)
1. Isolate and shear chromatin mechanically
2. Add antibody specific for acetylated N-terminal histone tail
3. Antibody against acetylated histone N-terminal tail
4. Nucleosome with acetylated histone tails
5. Immunoprecipitate
6. Release immunoprecipitated DNA and assay by PCR

(reverse X-links)
Analysis of histone modifications at the IFN-β promoter after viral infection

1. How was this ChIP experiment performed?
2. Which modifications happen first?
3. Name all the modifications that precede transcription.
4. Which modifications are not essential for transcription initiation?
5. At what time point does the pre-initiation complex form?
6. What does the state of histone acetylation prior to viral infection tell you about this promoter?

Chromatin-remodeling

Helicase activity - dissociate DNA from nucleosomes

How are chromatin remodeling factors recruited to specific genes?

Mediator Complexes:
- bind Pol II
- bind activation domains
- histone modification

Multiple Activators affect Mediator and General Transcription Factor Association with the Promoter
Pre-initiation Complex Formation

1. Condensed chromatin
2. SWI5
3. GCN5

Pre-initiation Complex Formation (cont.)

1. GCN5
2. SBF (activator)

Cooperative Interactions - Cell Specificity

Transthyretin (TTR) - secreted from liver to transport thyroid hormone

1. How would you test:
   1. The chromatin structure of TTR in hepatocytes versus fibroblasts
   2. The size of the mRNA expressed from TTR
   3. Which promoter sequences are required for transcription
   4. The specific DNA sequences bound by the HNF activators
   5. If there is sequential binding of the activators