Complex Transcription Machinery

Subunits of the Basal Txn Apparatus
Stepwise Assembly of the Pre-initiation Complex
Interplay of Activators, Co-regulators and RNA Polymerase at the Promoter
Purification and Cloning of the Basal Factors
Divide and conquer: biochemical identification of general transcription factors (GTFs) required for accurate initiation

Human cell nuclear extract

GTFs + RNA pol II + Cloned promoter DNA

Specifically-initiated mRNA
Basal ('General') Transcription Factors for RNA Polymerase II

- **TFIID** – consists of TBP (TATA-box binding protein) + TAFs (TBP-associated factors). Binds to core promoter motifs. TAFs interact with activator proteins. The first step in basal transcription is probably binding of TFIID to the core promoter.

- **TFIIB** – one subunit of 35 kDa. Binds to TBP and the BRE.

- **RNA Polymerase II** – consists of two large subunits (IIa and IIb) as well as about eight smaller subunits. Unique feature of largest (IIa) subunit is the C-terminal domain (CTD), which is an imperfectly-repeated heptapeptide motif, YSPTSPS.

- **TFIIF** – also known as RAP30/74. Binds to RNA polymerase II. Two subunits of 30 and 74 kDa. Functions in transcription initiation and elongation.

- **TFIIE** – two polypeptides of 34 and 56 kDa. Required for assembly of TFIIH into the transcription preinitiation complex (PIC).

- **TFIIH** – nine polypeptides. Core TFIIH has six subunits, which include 5'→3' and 3'→5' DNA helicases, and is also involved in nucleotide excision repair. Also has a three subunit Cdk7/MO15 + Cyclin H + MAT1 kinase complex that phosphorylates Ser5 of the CTD during transcription initiation.
Sequential assembly of the pre-initiation complex (PIC) and open complex by RNA polII + GTFs

TFIID = TBP + ~12 TAFIIIs

TFIIB 1 subunit

TFIIF 2 subunits

RNA pol II 12 subunits

TFIIE 2 subunits

TFIIFH 12 subunits - Has helicase and kinase activity
TBP (TATA-binding protein)

- Conserved C-terminal domain
- Dyad symmetry
- Binds multiple transcription proteins
- Binds in the minor groove and significantly bends DNA

*Figure 25-13. The X-ray structure of TATA-binding protein (TBP) from the plant *Arabidopsis thaliana*. [Courtesy of Stephen Burley, The Rockefeller University]*
Discovery of TAFs - Evidence for Co-activators

NHR

HSF

distal Enhancer

Proximal promoter

Sp1

SREBP

IIA

IID

TATA

IIB

IIE

IIF

IIH

Pol II Complex

110

250

60

DPE
DISCOVERY OF TRANSCRIPTION CO-ACTIVATORS: TAF Subunits of TFIID
Multiple Functions of TAFs: A Novel TAF-Acetylated Histone Tail Interaction

TAFs can serve as:
- Core promoter recognition factors
- Activator Targets
- Enzymes that modify proteins
- Chromatin-targeting modules via diacetylated histone tails
Three-dimensional structure of the human TFIID-IIA-IIB complex.

Position of IIB and IIA on the TFIID structure and mapping of the TBP. The blue mesh corresponds to the holo-TFIID, with the A, B, and C lobes indicated. (A) The green mesh corresponds to the density difference between the holo-TFIID and the TFIID-IIB complex. (B) The magenta and green meshes show the density difference between the holo-TFIID and the trimeric complex TFIID-IIA-IIB. The density depicted in light green can be attributed to TFIIB by comparison with (A), and the magenta density therefore corresponds to IIA. (C) The yellow mesh shows the density difference between the holo-TFIID and TFIID that is bound to the TBP antibody.
Tissue and Gene-Specific Core Promoter Recognition Complexes

Ubiquitious

Tissue-selective

Gene-specific
A Profusion of Coactivators, Cofactors, and Adaptors Contribute Additional Layers of Regulatory Specificity to Transcriptional Activation
CRSP Conformation is Activator-Dependent

Bound Activator

- VP16
  - leg: 145 Å
  - body: 360 Å
  - head: 150 Å

- None
  - 180° rotation
  - 90° rotation
  - leg: 130 Å
  - body: 300 Å

- SREBP
  - 180° rotation
  - 90° rotation
  - leg: 165 Å
  - body: 305 Å

Conformational Conversion:

Flag-CRSP-SREBP ← Flag-CRSP ← Flag-CRSP-VP16
Four activators enriched in hepatocytes plus the ubiquitous AP1 factor bind to sites in the hepatocytespecific enhancer and promoter-proximal region of the TTR gene.

The activation domains of the bound activators interact extensively with co-activators, TAF subunits of TFIID, Srb/Mediator proteins, and general transcription factors, resulting in looping of the DNA and formation of a stable activated initiation complex.
Activators work in part by recruiting components of the transcription machinery.
Combinatorial possibilities due to formation of heterodimeric transcription factors.

(a) In the hypothetical example shown, transcription factors A, B, and C can each interact with each other, permitting the three factors to bind to six different DNA sequences (sites 1–6) and creating six combinations of activation domains. (Note that each binding site is divided into two half-sites, and that a single heterodimeric factor contains the activation domains of each of its constituent monomers.)

(b) When an inhibitory factor (green) is expressed that interacts only with factor A, binding to sites 1, 4, and 5 is inhibited, but binding to sites 2, 3, and 6 is unaffected.
Inhibition by steric mechanisms

In the three mechanisms shown, the repressor either inhibits activation or directly interferes with formation of the initiation complex. In addition, some repressors interact with “co-repressor” proteins, that are thought to interact in turn with general transcription factors to inhibit initiation.

Later, in the chromatin section, we will consider another repression mechanism involving histone modification.
Inhibitory regulation by truncated HLH proteins.

The HLH motif is responsible for both dimerization and DNA binding. On the left, an HLH homodimer recognizes a symmetric DNA sequence. On the right, the binding of a full-length HLH protein to a truncated HLH protein that lacks the DNA-binding helix generates a heterodimer that is unable to bind DNA tightly. If present in excess, the truncated protein molecule blocks the homodimerization of the full-length HLH protein and thereby prevents it from binding to DNA thus behaving as a dominant negative regulator.
Major Points

1. Fractionation, identification and purification of the basal trxn machinery: TFIIA,B,D,E,F, H and RNA Pol II
2. Stepwise assembly of the pre-initiation complex (PIC) at RNA pol II promoters
3. TBP, a subunit of TFIID, binds core promoter and bends DNA
4. TFIID is composed of TBP and TAFs which bind promoter DNA and serves as a Co-activator to help link activators to the PIC
5. Metazoans evolved the use of TFIID and Co-regulators that contain cell-type or tissue specific subunits
6. Activators can induce large conformational changes in the structure of Co-activators
7. Combinatorial regulation mediated by: heteromeric activators, activator/repressor interactions and dominant negative molecules
8. Eukaryotes use a plethora of co-regulators (mediators, co-activators) to direct RNA pol II transcription