

**linker scanning:** make a new mutant for each 10 bp stretch (switch out those 10 nucleotides); observe phenotypic effects

**reading assignment:** Kostrewa et al, Nature 2009

- B reader: identify TSS
- B linker: open (melt) promoter
- H: helicase and kinase activity
- F: bind RNAPolIII, interact with non-template strand
- H: phosphorylates RNAPolIII CTD (C-terminal domain)
- E: stimulates H kinase activity
- S: stimulates proofreading and correction of transcripts

We will not cover TF classes I and III in detail.

## Function of TFIID

### TBP (TAT box-binding protein)

- binds TAT box at minor groove
- bends DNA to start transcription initiation

### TAF<sub>II</sub>s (TBP associated factors)

- recruit TBP to TATA-less promoter to start transcription initiation (TAF<sub>II</sub>250 and TAF<sub>II</sub>150 bind to initiator and DPE, TAF<sub>II</sub>250 and TAF<sub>II</sub>110 interact with Sp1 that binds to GC box)
- different TAF<sub>II</sub>s are required to respond to various activators
- TAF<sub>II</sub>250 has histone acetyltransferase (HAT) and kinase activities that modify chromatin and other transcription factors

## Promoter proximal pausing

- pause sites 20-50bp downstream of TSS
- two proteins help stabilize RNA PolII in paused state: DSIF and NELF
- P-TEFb delivers signal to leave paused state