BICH/GENE 431 KNOWLEDGE OBJECTIVES

Chapter 12 - Transcription

Basics: 5' to 3', transcription bubble (~14 nt), template vs. nontemplate strands, no primer needed, start site (+1), promoter, upstream vs. downstream

Transcription cycle

- initiation: closed complex, open complex, initial transcribing complex
- elongation (follows promoter escape)
- termination

E. coli RNA polymerase – core enzyme vs. holoenzyme, know subunits

Structures of bacterial promoters: -35 and -10 elements, UP element, start site usually a purine

Consensus sequences for promoter elements

What parts of E. coli RNAP bind what promoter elements?

Transcriptional initiation at bacterial promoter

- closed complex described by equilibrium constant (K_D)
- open complex formation is irreversible, described by rate constant k_2
- abortive initiation: what is it? Possible models (scrunching model is favored).
- promoter escape

Different channels in E. coli RNAP: main entry channel for DNA, nucleotide channel, RNA exit channel, template and nontemplate strand channels

Transcriptional proofreading with E. coli RNAP

- pyrophosphorylytic editing: simple reversal of polymerization reaction
- hydrolytic editing: hydrolysis of one or two nucleotides; stimulated by Gre factors

Termination in E. coli

- rho-dependent: rho factor, ATPase, binds rut sites in RNA
- rho-independent (or intrinsic): no additional proteins needed; know structure of terminator in DNA and RNA and possible model how it works

Three eukaryotic RNA polymerases and what types of genes they transcribe

Multiple subunits for RNA polymerases – understand RPB, RPA, RPC nomenclature; some common subunits; 3D structures of eukaryotic and bacterial RNAPs similar

Eukaryotic promoters: core promoter, regulatory elements (proximal, upstream, enhancers)

Possible core promoter elements for RNAPII protein-coding genes – mix and match: TATA box, Inr (initiator), downstream elements (DPE, DCE), BRE (TFIIB recognition element)

What general transcription factor binds what core element?

Know general transcription factors (GTFs) and what they do in formation of preinitiation complex at the promoter (TFII terminology)

- TFIID composed of TBP (TATA box-binding factor) and TAFs (TBP-associated factors)

  TBP binds minor groove with beta sheets and bends DNA about 80 degrees
- TFIIA assists TFIID binding
- TFIIB binds next and establishes directionality for transcription
- TFIIF associates with RNAPII and ushers to promoter

- TFIIE assists binding of TFIIH
- TFIIH has many subunits and crucial functions: helicase activity, kinase activity, nucleotide excision repair proteins

CTD (carboxy terminal domain) of RNAPII
- on largest subunit (RPB1)
- heptapeptide repeat sequence many times
- several possible phosphorylation sites on CTD that serve regulatory functions
- no phosphorylation on CTD for RNAPII that binds to promoter
- Serine5 of CTD phosphorylated by TFIIH kinase correlated with promoter escape

Mediator complex necessary for transcription by RNAPII in the cell – many subunits (MED subunits); serves as a conduit between activator proteins and the preinitiation complex; interacts with CTD of RNAPII

Role of CTD phosphorylation in elongation by RNAPII: P-TEFb kinase phosphorylates on serine2 is signal for elongation

Elongation factors stimulate elongation by RNAPII: P-TEFb, SPT5, ELL proteins, TFIIS

TFIIS stimulates hydrolytic editing (proofreading) by RNAPII

FACT (Facilitates Chromatin Transcription) is protein complex that promotes RNAPII transcription through nucleosomes – binds H2A/H2B dimers to remove ahead of polymerase and puts them back on afterwards.

Basic structure of pre-mRNA and mRNA in eukaryotes: 5' cap, 5'UTR relative to start codon, exons and introns, 3'UTR relative to stop codon, polyA tail on 3' end

5' capping
- capping enzymes associate with RNAPII when serine5 of CTD is phosphorylated via Spt5 protein
- be able to draw structure of 5' 7-methyl-cap showing all atoms
- RNA triphosphatase, guanylyltransferase, methyl transferase enzymes
- functions of 5' cap

Polyadenylation reaction
- before termination of transcription
- CstF and CPSF proteins
- Cleavage of RNA and addition of polyA tail by polyA polymerase (PAP)
- ~200 A residues in tail
- functions of polyA tail
- ability to isolate mRNA from total RNA using affinity chromatography with oligo(dT) or oligoU columns

Two models for termination of RNAPII transcription
- torpedo model uses ribonuclease Rat1(yeast)/Xrn2(human)
- allosteric model hypothesizes conformational change in RNAPII after cleavage/polyadenylation makes it more susceptible to termination

RNAP I promoter: only one gene for 18S, 28S, 5.8S ribosomal RNAs, but present in many copies

Core promoter and upstream control element (UCE)

Core promoter bound by SL1 – contains TBP and TAFs specific for SL1

UCE bound by UBF (upstream binding factor)

3 types of RNAP III promoters in metazoans
- Type 1: 5S rRNA genes with box A and box C elements within transcribed region

- Type 2: tRNA and other genes with box A and box B elements within transcribed region (called ICR: intragenic control region)
- Type 3: U6 snRNA and other genes with external promoters containing many elements found in RNAP II gene promoters; usually no ICR

ICR is characteristic of many RNAP III gene promoters – tRNA gene box A and box B bind TFIIIC protein; this recruits TFIIIB protein, then RNAPIII

TBP is a component of general transcription factors for almost all eukaryotic promoters, irrespective of type (only exceptions when a TBP-related protein is used instead).