2. Double helix needs to be melted apart
3. RNA synthesis is initiated = no primer is required
4. Extending the RNA molecule 5' → 3'
5. Modify the RNA
6. Terminate the RNA molecule

Prokaryotic RNA polymerase has multiple subunits that come together to form the complex

Initiation

Promoter regions direct RNA polymerase to transcription start sites

Prokaryotes: Sigma subunit of RNA polymerase interact with -10 and -35 promoter regions

-10 means 10 bases upstream from the TSS

Upstream elements (UP) that are 40-60 nucleotides upstream of the TSS

Bound by alpha subunit of RNA polymerase = increases efficiency of RNA polymerase = creates additional binding site for the polymerase

Eukaryotes: TATA box is the promoter region

If the sequence matches the TATA box more similarly, stronger transcription at that gene

Eukaryotes:

Polymerase unwinds 17 base pairs of DNA to make a bubble (open promoter complex)

Energy from ATP or GTP is needed to promote transcription to occur → why the first base is always an A

Elongation

Prokaryotic or Eukaryotic:

1. DNA template
2. Activated precursors = ribonucleotide triphosphates
3. Divalent metal ion = magnesium or manganese
    a. Helps form structure of polymerase

RNA polymerase = 50 base pairs/second

- Some proofreading activity, not very picky
    - Doesn't care as much because if transcript isn't perfect it can be degraded and another one can be made
- DNA polymerase works harder to make sure DNA polymerase is more perfect = 1000+ bp/sec

Transcription is slower and more error-prone than DNA replication.

Nuclear Receptor Regulation of Expression

TFs play an important role

Estradiol comes in and binds to nucleoreceptor = changes conformation of protein = dimerization = recruitment of co-activators

Co-activators such as HAT can be recruited to add even more acetylation to chromatin to make it more accessible

-coactivators work with other proteins to form large complexes that modify chromatin and the transcription machinery to regulate gene expression

Drugs that mimic hormones and toxins can activate or repress receptors

Chapter 38: RNA Processing in Eukaryotes

Processing of Eukaryotic rRNA

RNA Polymerase I transcription results in a single precursor (45S in mammals) that encodes three RNA components of the ribosome:

- 18S rRNA
- 28S rRNA
- 5.8S rRNA

18S rRNA is the RNA component of the small ribosomal subunit (40S)

28S rRNA + 5.8S rRNA are the components of the large ribosomal subunit (60S)

Nucleotide modification = methyl groups are added to nucleotides, conversion of uridine to pseudouradine. Forms a big ribonucleoprotein complex → will cleave out the parts that are the final rRNA

Processing of Eukaryotic tRNA

tRNA = bring amino acids to ribosomes during translation

Complex structure of tRNA = palindromic sequences so they can fold back

1. Leader and trailer sequences are cleaved off
2. Intron in tRNA will be spliced out (not all)
3. Modification of some nucleotides
    a. Pseudouradines, methylation of bases, etc.
4. Addition of AA attachment site
    a. Polymerase comes in and adds amino acid attachment site

b. Provides site for the amino acid to be attached to tRNA in order for tRNA to bring amino acid to ribosome for translation

Ins and Outs of Eukaryotic mRNA

Within transcribed region, coding sequence and non-coding sequence

Within RNA coding regions are exons

Introns do not code for protein and are spliced out

premRNA = non-spliced

mRNA = spliced and used for translation

Poly A tail

UTR = this transcript is translated into protein

Untranslated region = going to be something upstream of the start codon; will be some transcript left after the stop codon

5' Cap

RNA not require a polymer

First base has all of it's phosphates

5' triphosphate = increased stability, protecting from phosphatases and nucleases

Linked to translation of mRNA

RNA has 3 phosphates at the 5' end:

1. First phosphate will be hydrolyzed and removed
2. GTP will be added to that 5' end
    a. First phosphate of GTP will attack diphosphate at 5' end
    b. Links GTP to downstream mRNA
    c. Opposite direction
3. Methylation can happen = N-7 of guanine methylated
    a. If only methylation, called the 0 cap
    b. Can also be methylation within first bases of RNA
        i. First base methylated = 1 cap
        ii. First and second base methylated = 2 cap

Poly-A Tail

AAUAA= termination

Transcript that has a 5' cap and towards the end it has the AAUAA sequence

White color = insert is in the middle of the Lac Z gene, just isn't being expressed

The Story of Insulin

Early 1900s = patients ingested animal pancreas

Insulin isn't orally available = broken down in GI tract = won't get to receptors

1922 = injects dog insulin into Type I diabetes patient

It did help = but not ideal because it could cause an immune reaction

Not really as purified as much as it should have been or could have been

1978 = synthetically manufactured human insulin

Used bacteria = cloned human insulin gene into bacterial plasmid = took it out, purified it

1980s to current = availability of human insulin revolutionized the treatment of diabetes

How Exactly did They do This?

Human pancreas tissue or any human tissue = insulin is in every cell of the body

Can't use genomic sequence to clone into bacteria = NEED TO USE RNA

1. Take RNA from pancreas (doesn't have introns)
2. Need to make cDNA
3. Clone cDNA into recombinant plasmid
4. Transform this into bacteria
5. Using a bacteria promoter, tell bacteria to transcribe this gene and it will
6. Bacteria will make that protein and it can be purified

Reverse transcriptase = makes complementary DNA from RNA template = yields cDNA

1. Won't contain uracil
2. Deoxynucleotides

RNA isn't very stable → why it can't be cloned

Has to be double stranded, cut with RE, must have a sticky end

cDNA Synthesis and PCR

All mRNA has a poly-A tail

Utilize this to our benefit… reverse transcriptase needs a primer

Put in an oligoT primer = binds to poly-A tail

Reverse transcriptase will start reverse transcribing the mRNA

Enzyme goes backwards on mRNA and adds complementary nucleotides to original primer that's there

One strand is RNA… one strand is DNA → separate the two strands and use as template for PCR