Where is RNA synthesized?
pulse chase expt with hot uracil showed: (P 297)
RNA first appears in nucleus, then in cytoplasm.

Is one strand of DNA used? Expt by Marmur:
SP8viral DNA separated on CsCl2 gradient: ( p 300)
separated DNA strands, cooled rapidly
mRNA hybridizes (binds) only to one strand.
Template strand is termed the sense strand.

TRANSCRIPTION: (p 300) http://www.youtube.com/watch?v=WsofH466lqk
Two regions of homology in all promoters (consensus sequences):
at -35 and -10 (-10 is Pribnow box: TATAAT):

initiation
-35: RNA holoenzyme polymerase (tetramer, 2 α, 2 β) scans DNA, stops at Promotor: p 302
-10: Then sigma (promotor recognition, initiation factor) in holoenzyme, unwinds DNA at -10 region forming open
promoter complex. Sigma dissociates once elongation begins. (p 307)

elongation
5′ to 3′, similar to DNA synthesis (note the untranslated 5′ length upstream fr AUG)

termination, two types: (p 303, 304)
1) GC rich region in RNA forms hairpin loop, followed by U rich region dissociates
2) rho factor for release (NusA protein) attaches to rut site, (rho utilization site) pulling RNA off the polymerase
using ATP hydrolysis to drive rxn

three types of ribosomal RNA by sucrose gradient: 5S, 16S, 23S

EUKARYOTIC RNA (p 304)
2/23/94, 2/23/96, 24 Feb 97, 20 Feb 08

in eukaryotes, three RNA polymerases,
RNA polymerase I synthesizes r RNA
RNA polymerase II synthesizes mRNA (monocistronic) produce RNA transcripts (once called heterogeneous nuclear RNA)
polymerase III synthesizes tRNA

Initiation: TATA-binding protein binds to TATA box, recruits other transcription factors
RNA polymerase II binds to “start” site.
Carboxyl Tail Domain (CTD):
a protein tail which is phosphorylated, facilitates post
transcriptional processing

Co-transcriptional processing: (p 308)
cap at 5′ end: begins with guanosine, methylated to form 7 methylguanosine via tri-PO4 bond
tails added to 3′ end: poly A after transcription (stabilizes transcript)

RNA splicing to remove internal portions of transcript

Split genes: introns intervening sequences must be removed and
exons coding regions must be joined to produce message

small nuclear ribonucleoprotein (snRNP) particles perform editing (‘ribozyme activity’)

Lariat model uses a snRNP to recognize --AGGU----AGGU-- (start, stop)
to clip out (p 311) http://www.youtube.com/watch?v=4X8eK15R8yY

exon shuffling allows interchange of domains in proteins, more rapid
evolution of proteins (p 309)