6. RNA SPLICING II

6.1. SPLICING PROOFREADING, INTRODUCTION

see ch 5.12, 5.13, pg. 5.3

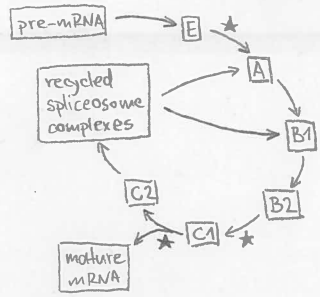
DEAD-box proteins ch 5.13, pg. 5.3

- number of steps in spliceosome cycle involve RNA-RNA pairing and its changes → ATP needed to speed up RNA-RNA pairing changes
- ATP hydrolysis
  - speeds-up splicing ↑
  - contributes to overall fidelity of splicing

see ch 5.9, pg. 5.2

sites that can be mistaken for real ones due to similar sequence

- signal identifying branch point and 5'/3' splice sites is weak → many possible cryptic sites throughout pre-mRNA
- adding ATP-dependent steps into spliceosome cycle increases fidelity ← ensures use of correct sites

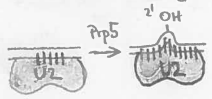


★: ATP hydrolysis (see ch 5.12, 5.13, pg. 5.3)

ensures unidirectionality of spliceosome cycle

6.2. SPLICING PROOFREADING, PRP5 AND PRP16

- Prp5
  - interacts early in splicing reaction: E → A (see ch 6.1) (see ch 5.12, 5.13, pg 5.3)
  - interacts w/ U2
    - U2 forms several strong H-bonds w/ branch point → Prp5 strengthens U2/branch point interaction



Prp5 helps U2 to form A complex, only if U2 already interacts well w/ pre-mRNA

    - U2 does NOT form strong H-bonds w/ branch point → Prp5 does NOT act on U2 → U2 more likely to fall of the pre-mRNA

- Prp16
  - interacts late in splicing reaction: C1 → C2 (see ch 6.1) (see ch 5.13, pg 5.3)
  - checks if the cleavage of 5' splice site by the brang point has occured
  - helps to position 3' end of spliced exon to 3' splice site, so that C2 complex can be formed

Prp16 helps to form C2 complex, only if the lariat was correctly formed

6.3. SPLICING PROOFREADING, ATP-DEPENDENT REACTIONS

E: enzyme E': enzyme in a lower energy state (different RNA conformation, exciting of one of splicing factors, ...) S: substrate P: product

$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E+P$ } "good pathway"

$k_3 \| k_3$

$E+S$ } "wrong" substrate can enter the pathway

$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E'S \xrightarrow{k_3} E+P$ } "good" pathway

$E'S \xrightarrow{k_4} E'+S$ } "wrong" substrate can NOT enter the pathway

extra step that consumes energy (eg. ATP hydrolysis)

only high-energy state enzyme forms ES complex rapidly

- example: Prp16

$L1 + Prp16\text{-}ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} L1 \cdot Prp16\text{-}ATP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} L1^* \cdot Prp16\text{-}ATP$

$L1 \cdot Prp16\text{-}ADP$ (lariat does NOT form)

$L1^* \cdot Prp16\text{-}ADP$ (correct cutting occurs & lariat forms)

lower energy state (ADP) → can not return back
- correct product must continue down the pathway (cannot return back)
- incorrect product can not re-enter the pathway (cannot return back)

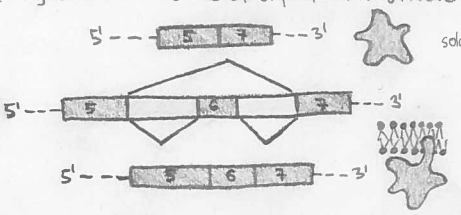
L1: Lariat Intermediate
L1*: Lariat Intermediate following conformational rearrangement

see ch 5.13, pg. 5.3 (intermediate step)

6.4. Alternative Splicing, Pt. I

- alternative splicing: common especially in higher eukaryotes - e.g. human average gene ┬ 7-8 exons
  constitutive: making a family of related proteins └ ~3 alternative isoforms of mature mRNA
  regulated: e.g. different versions of a protein in different tissues

- eg.



6.5. Alternative Splicing, Pt. II

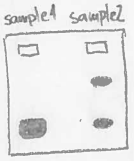
- gene number paradox: higher organisms expected to have higher number of genes ← they may have higher number of proteins, but more proteins are expressed from the same genes in higher organisms, hence relatively lower # of genes

- grape: 30'434
- human: 22'333
- chicken: 16'736
- fruitfly: 14'893
- E. coli: 4'143
- Influenza: 11

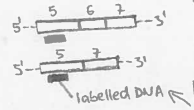
} number of protein-coding genes

- detection of alternative RNA splicing: northern blot
  - process
    - separate mRNA by gel electrophoresis
    - transfer RNA to membrane
    - hybridize labelled DNA probe to RNA

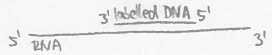
getting old-fashioned



need MATURE mRNA → extracted from CYTOPLASM (not from nucleus) !



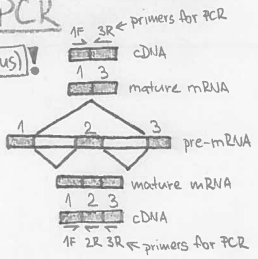
can hybridize w/ different isoforms of the gene encoded in the genome → need sequence long enough to be unique !

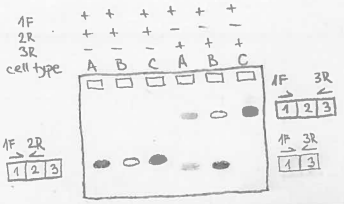


Reverse Transcriptase ← cDNA →

6.6. Assays for Alternative Splicing, RT-PCR

- process
  - isolate RNA from cells/tissues of interest ← mature mRNA (from cytoplasm, not nucleus) !
  - make cDNA
  - amplify a region of interest by PCR and quantify the amount of product
    - by radioactive PCR and gel electrophoresis
    - or, by qPCR (a.k.a. real-time PCR)

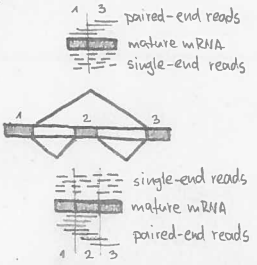


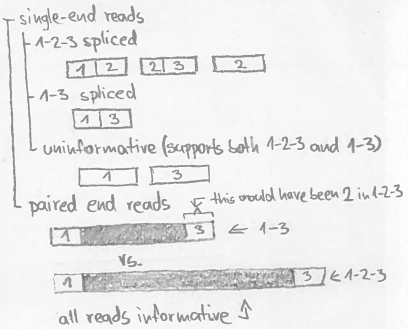


- caveats
  - primers may not hybridize to different places on RNA w/ the same efficiency
  - PCR reactions may not be equally efficient for all cases (e.g. sequences diff. to replicate)

6.7. Assays for Alternative Splicing, RNA-seq

- process
  - isolate mature mRNA ← mature mRNA from cytoplasm, not nucleus
  - make cDNA
  - fragment
  - size-select (so that size of sequenced fragments is known)
  - perform high-throughput sequencing on particular size-range of fragments





6.8. Importance of Alternative Splicing in Metazoans

Kingdom Metazoa a.k.a. Animalia

- human
  - # of protein coding genes: 22'180
  - % of genes w/ 2+ isoforms: 88%
  - avg. isoforms per gene: 3.4
  - total # of isoforms: 215'170

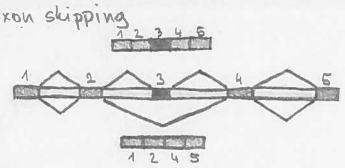
} 10x ← all genes, including those not coding for proteins

- D. melanogaster (fly)
  - # of protein coding genes: 13937
  - % of genes w/ 2+ isoforms: 45%
  - avg. isoforms per gene: 1.9
  - total # of isoforms: 29'173

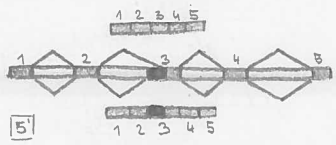
} 2x

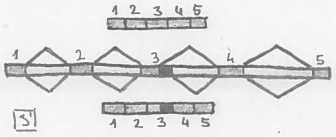
6.9. Mechanisms of Alternative Splicing

- exon skipping

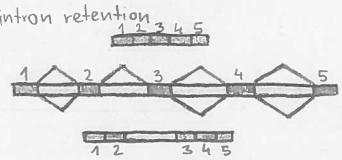


- alternative 5'/3' splice site

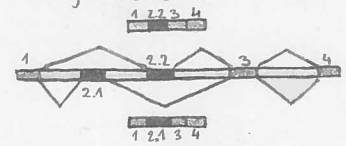




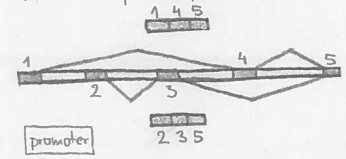
- intron retention

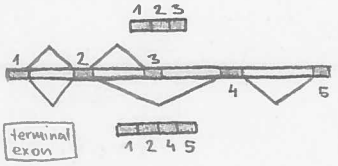


- mutually exclusive alternative exons



- alternative promoter/terminal-exon

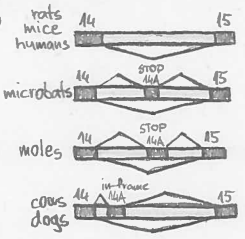


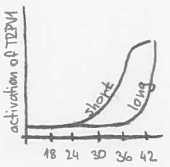


6.10. Alternative Splicing Examples

- troponin T
  - α troponin T (adult): exons 1,2,3,5
  - δ troponin T (fetal): exons 1,2,4,5
  - } see mutually exclusive alternative exons (ch. 6.9)
  - only 14aa difference: affects way of troponin T interaction w/ tropomisin ⇒ type and mechanism of muscle contractions slightly different
- vampire bats - obligate consumers of blood from warm-blooded organisms (sole food source)
  - need a way to sense warm-blooded animals / part of the body where blood may be easily available
  - alternative splicing of common thermoreceptor (sensory ion channel TRPV1)
  - "normal" form
    - present in many animals (sensory neurons)
    - allows sensing dangerous heat levels (above ~43°C)
  - short form
    - present only in vampire bats (sensory neurons of ganglion pits)
    - allows sensing blood temperature
    - missing ~63aas from C-terminus

specialized organ around nose of vampire bats





6.11. Genomic Introns and Exons

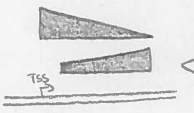
← cartoon of exons/introns more to-scale w/ reality

- exons - usually 50-250 nt, rarely longer than 500 nt
  - often encoging just a single domain of protein, region encoding different specificity, ...
- introns - usually ~1000 nt long, can be 1000s nt long

		human	D. melanogaster
exon	mean	320 nt	494 nt
	median	145 nt	272 nt
intron	mean	7563 nt	2068 nt
	median	1964 nt	642 nt

6.12 Defining Splice Sites

- loading of splicing machinery
  - cooperating on splicing
  - exon1 — U2 — exon2
  - loaded together
  - tail of RNA Pol interacts w/ splicing factors
    - during txn
    - Ser5-Pi : capping, ...
    - Ser2-Pi : recruitment of RNA splicing factors

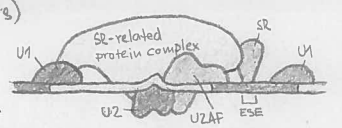


see MITx 7.28.2x pg 9.1
Ser2-Pi, RNA Pol CTD
C-Termined Domain (tail)

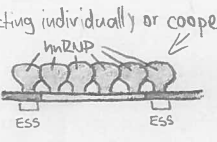
6.13. Regulation of Splicing, Enhancers and Repressors

can promote usage of weaker splice sites in favor of stronger ones

- 2 protein families: bind to small redundant sequences accross pre-mRNA (both introns and exons)
  - SR proteins
    - act positively: help splicing factors to interact w/ splice sites (promote/enhance binding of splicing factors)
      - + co-activator model
    - binding sites
      - ESE (Exonic Splicing Enhancer)
      - ISE (Intronic Splicing Enhancer)
  - hnRNPs
    - act negatively: sterically inhibit binding of splicing factors, acting individually or cooperatively
    - binding sites
      - ESS (Exonic Splicing Silencer)
      - ISS (Intronic Splicing Silencer)

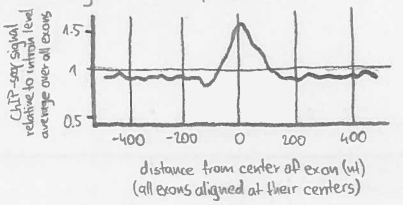


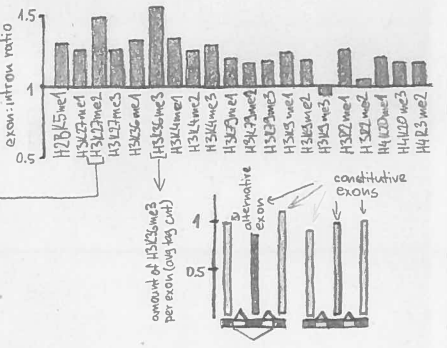
co-activator model



6.14. Effect of Chromatin on Splicing

- chromatin state of the template that is being transcribed
  - slow down transcription ⇒ splicing factors have more time for assembling on nascent pre-mRNA
    - mediated by Ser2-Pi on RNA Pol CTD - see ch. 6.12
  - (help) recruit additional splicing components
    - activators
    - repressors
  - ! Cell-type-specific !

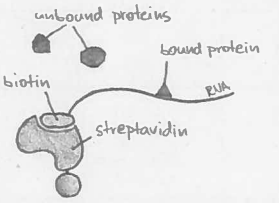




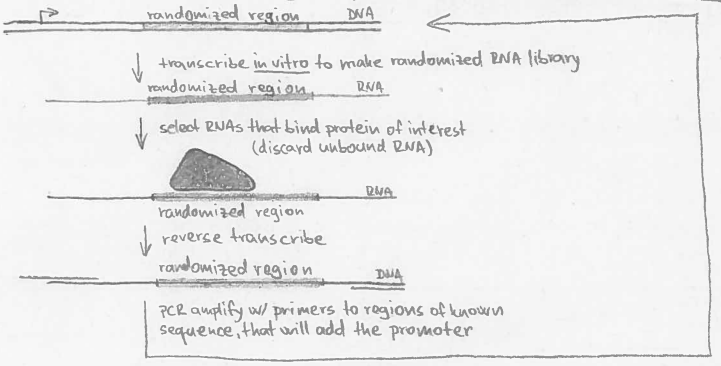
6.15. Assays for Regulation of Splicing

- EMSA - e.g. add protein at different concentrations (in different lanes) to small amount of radioactively labelled RNA and observe different amounts of shift at different protein concentrations

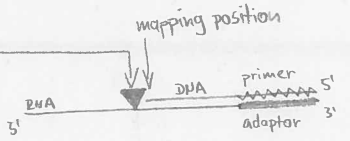
- bead-mediated RNA affinity chromatography
  - process
    - isolate RNA of interest
    - biotinylate the RNA
    - incubate w/ cell extract containing all factors needed for reaction of interest
      - proteins will bind RNA
    - capture RNA using streptavidin
    - identify proteins bound to RNA
      - western blot
      - mass spectrometry (need large enough amount of captured protein)
        - new splicing regulators can be discovered using this type of assay



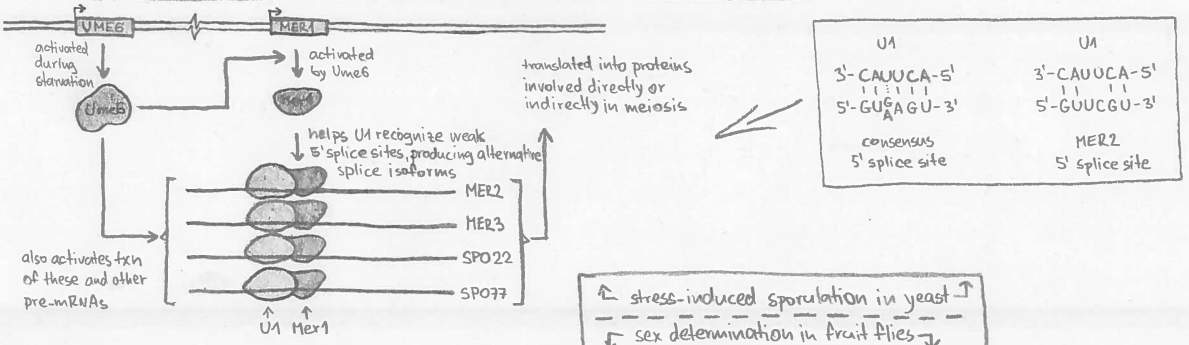
- SELEX (Systematic Evolution of Ligands by EXponential enrichment) ← see 7.28.2x, pg. 10.1



- iCLIP-seq : maps position of protein binding to RNA
  - takes advantage of the fact that RNA absorbs UV light very well ⇒ easy to covalently crosslink bound proteins
  - process
    - UV irradiate cells to crosslink protein to RNA
    - make lysate
    - partially RNase digest into fragments
    - immunoprecipitate RNA-protein complexes containing protein of interest
    - digest protein w/ Proteinase K, leaving only peptide crosslinked to RNA
    - ligate 3' adaptor to RNA, generating priming site
    - reverse transcribe to make cDNA - cannot get past the peptide on RNA
    - prepare a sequencing library from cDNA
    - high-throughput sequencing
    - map to genome

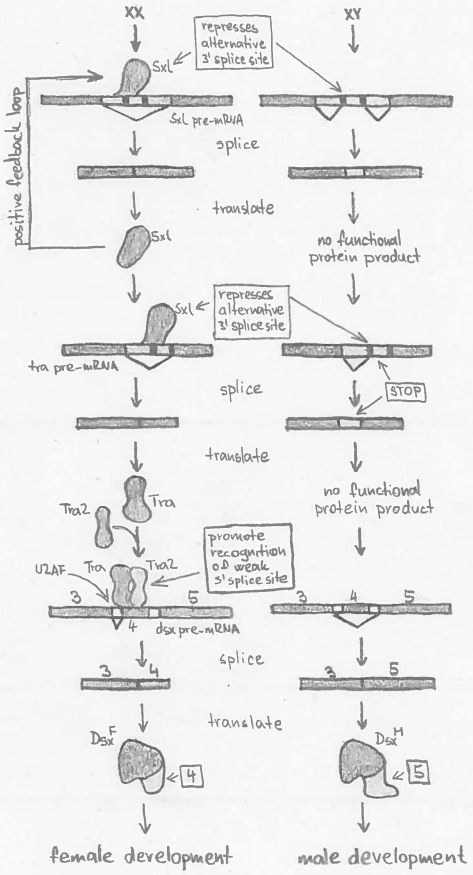


6.16. Examples of Regulation of Splicing



↑ stress-induced sporulation in yeast ↑

↓ sex determination in fruit flies ↓



6.17. Conclusion

ooo (see ch.5, ch6.)