

BICH/GENE 431 KNOWLEDGE OBJECTIVES

Chapter 13 – RNA Splicing

Basic terminology: exon, intron, 5' splice site, 3' splice site

Ave # of introns per gene increases from single-cell eukaryotes up through mammals

In humans, ave. size of exon is 150 nt and ave. size of intron is 3000 nt

Discovery of introns in eukaryotic genes in adenovirus (P. Sharp laboratory, MIT)

R-loop mapping, spliced tripartite leader sequence on all late mRNAs

Eukaryotic mRNAs are transported to cytoplasm after processing (cap addition, polyadenylation, splicing) – regulated through nuclear pore complexes

Conserved sequences mark important features of introns

- GU at 5' splice site for major type of splicing
- AG at 3' splice site for major type of splicing
- Branchpoint A residue near 3' splice site
- Polypyrimidine tract between branchpoint and 3' splice site

Two transesterification reactions during nuclear pre-mRNA splicing

- be able to draw atomic structure of splicing branchpoint
- release of lariat intron and spliced exons

Spliceosome

- snRNPs (snurps): U1, U2, U4, U5, U6 snRNAs + tightly associated snurp proteins
- plus many other proteins to assist and regulate splicing
- be able to draw splicing pathway using snurps and BBP, U2AF proteins; identify various dynamic complexes
- know what basepairing interactions exist in spliceosome complexes between snRNAs and 5' splice site, branch point, and between different snRNAs (do not memorize exact basepairs)

SR proteins (serine/arginine-rich) bind exonic splicing enhancers and assist binding of splicing factors; help to mark exons (exon definition); also used for regulation of alternative splicing (later)

Minor spliceosome (less than 1% of splice sites) use AT-AC intron splice sites

- variant snRNAs (and snurps): U11, U12, U4_{ATAC}, U6_{ATAC}, use same U5

Types of alternative splicing: exon skipping, exon extended (alternative 5' or 3' splice sites), intron retained, mutually exclusive exons

Examples of alternative splicing

- troponin T gene: mutually exclusive exons
- SV40 virus T antigen gene: alternative 5' splice site usage
- sex determination in *Drosophila* (details later)

Possible mechanisms for regulation

- steric hindrance when intron is too small: both U1 snRNP and U2 snRNP cannot bind at same time to same intron; leads to mutually exclusive exons, like for troponin T
- combination of GU-AG and AT-AC splice sites in same pre-mRNA can lead to different combinations; but cannot splice together two different types of junctions
- splicing enhancers bound by activators or splicing silencers bound by repressors

repressors (example, hnRNP1 protein) block splicing sequences like polypyrimidine tract

activators recruit splicing factors to certain splice sites; many activators are SR proteins

Understand how sex determination in *Drosophila* is controlled by alternative splicing (Fig. 13-25): Sxl protein is splicing repressor, Tra protein is splicing activator, Dsx protein produced in male and female form and controls sex-specific transcription

Know some examples of human diseases controlled by RNA splicing defects

What is exon shuffling and why is it important in eukaryotes?

Self-splicing introns

- Group I and Group II; rare; found in some organelles in plants, fungi, simple eukaryotes, bacteria
- Group I rRNA intron that self-splices discovered by Tom Cech
- know basic chemistry of splicing of each type
- assisted by proteins in the cell

RNA editing: two general types: site-specific deamination or guide RNA-directed insertion/deletion of multiple U residues

Site-specific deamination: C to U by cytidine deaminase or A to I (inosine) by ALAR (adenosine deaminase acting on RNA)

Example of C to U in human apolipoprotein B gene, changes to stop codon

A to I deamination in ion channel protein expressed in brain; I base pairs to C, so codon containing I is different from A in same position

Guide RNA-directed editing is found in mRNA of trypanosomes (mitochondria)

- many insertions or deletions of U residues drastically changes mRNA sequence
- know basic outline of steps in this editing process (for insertions) using guide RNA, endonuclease cutting, U addition by TUTase (terminal uridylyl transferase), RNA ligase