Lecture 16

- Intron - anything removed from the primary transcript.
- Splicing - many RNAs when they are transcribed get processed at the 5' to 3' ends, by cleavages
  - removal of some sequences out of the middle of the entire sequence, splicing does not occur if the removal occurs at the ends of the sequence.
- Non-coding RNA - such as ribosomal tRNAs, etc all have introns in them. Introns can be located in the protein coding region if you have an mRNA or can be located in the 5' or 3' UTR, which doesn't affect protein expression. Although having introns in the UTR can make translation less efficient or may destabilize the RNA.
- Introns are any sequence of RNA that was removed which is present in the primary transcript that is not present in the final functional form of the RNA whether it is coding or non-coding
- Transesterification - exchange of phosphodiester bonds without the breakage of bonds, no energy required
- Cleavage - break a phosphodiester bond on both sides of the introns, then ligate the two pieces of exons back together requires ATP.
  - In cleavage and ligation in RNA the proteins are the enzymes
- RNA vs protein mediated - when have an enzyme mediated reaction, the enzyme provides two functions:
  - 3D structure that usually has a cavity that has a substrate binding site, and has catalytic functional groups that are in the vicinity of the bonds that have to be broken or reformed. The functional groups are usually on the side chains of the amino acids.
    - Protein site has a binding site, K_m, and a functional groups mediating catalysis
  - RNA medicated catalysis, the same principles apply, can apply Michealis-Menton kinetics to RNA enzymes
    - the RNA takes on a certain secondary and tertiary conformation. The base pairing of complementary sequences stems to create a secondary structure in the RNA, then base pairs in the unpaired loop regions. So when you have single stranded RNAs forming stems and loops, there are bases in the loops that can base pair to other bases in the loops which creates 3D folding structure. That structure has cavities that are substrate binding sites
    - The 2'OH on the ribose, provide the catalytic residues to mediate catalysis. The RNA enzyme has a binding site for substrates which generally are RNA. And provides the functional group that mediates the catalytic mechanism so it is the enzyme.
- Nuclear splicing there exist enormous numbers of proteins in this giant complex

    - the short stems that are involved in the secondary structure of the individual snRNAs or the snRNAs pairs the intron. Are not stable enough to survive in vivo conditions without proteins to stabilize them.
    - In RNA mediated events, it's not that the proteins don't play a role, its that the RNA provide the substrate binding sites for catalysis, without the protein the RNA probably wouldn't be functional in vivo.
- Group II introns can cause catalytic excision without any protein present, that is always with group I introns.
    - in vivo they require proteins for stability
    - The proteins do not provide a catalytic role, they are solely for stability
- There are many biological processes that utilize complexes of RNA and proteins together, some the RNA provides the catalytic residues and others its the proteins; it differs from process to process
- Which component of the ribosome, is it the rRNA or is it the ribosomal proteins that are responsible for mediating peptide bond formation?
    - It is actually the tRNA, it is thought that it is one of the Adenines in the terminal CAA of the tRNA that provides that catalytic residue. So its not even the rRNA or the ribosomal proteins. Its actually the tRNA that juxtapositions the other tRNA that mediates the catalysis.
- 3 of the 4 involved transesterification and direct joining one phosphodiester bond to another, the catalytic functional group is 2' OH on a ribose on one of the residues on the RNA
- The difference between autocatalytic splicing and nuclear splicing has to do with whether you do splicing in cis or trans
    - cis - regulatory sequences that act on the coding region attached to it
    - trans - bringing something in from outside so it can act on any substrate not on the one that produced it
- splicing - autocatalytic - all that you need for splicing is contained within the intron itself, so it acts cis on itself. Can't have an intron catalyze the extension of another intron on another piece of RNA, it paralyzes itself, so it acts in cis
- splicing - Nuclear RNA splicing - snRNAs that are coded all over the genome acting trans to mediate splicing with introns. Even though it is the same mechanism as group II autocatalytic splicing, it is now a trans splicing event. Even though the two ends of the intron are together that are usually recognized, that they require components from the outside to finish splices. snRNAs or snRNPs are an example of a trans acting factor. snRNPs are a trans acting factor in terms of nuclear splicing
- Fig 26. 18

- 95% of all introns use nuclear splicing, and require specific dinucleotides at both ends of the intron and A to the branch site that catalyzes two transesterification reactions: 1) 2'OH the A attacks the junction between the exon and the intron and 2) free 3'OH from the last base of the exon attacks the exon/intron junction.
- Group II splicing is the same as nuclear splicing except that they don't require snRNAs because the other components of the intron function in the same way as the snRNAs. Similar to nuclear splicing, there is the excision parts of the stem and loops making them independent U1 - U6 RNAs forming a complex that looks like the spliceosome. Have the same two transesterification reactions, A at a branch site attacks the junction , this junction attacks the other junction. End up liberating intron that now has the branch junction to form a lariat. Now end up with no ligated exon, but don't require a ligase, they are produced as part of the transesterification.
- Mitochondria and chloroplast genes have group I and II introns in them.
  - Some viruses of bacteria, bacterial genes also have group I and II introns
    - bacteria don't have nuclear splicing, they don't even have a nucleus.
    - Only splicing in prokaryotes is type I or II autosplicing.
- Within the same organism the same organelle introns of either group I or II in either in different genes.
- Group II introns are being lost so that most of the introns in lower introns are group I because if you have a group II intron and nuclear splicing in the same organism you can get internal deletions and have the group II being spliced using nuclear splicing machinery
  - i.e. nuclear splicing machinery deletes group II so the resultant method for splicing is nuclear splicing
- Alternative splicing is only found in nuclear splicing, you don't see it in autocatalytic introns, because the secondary and tertiary structure is dictating where the reacting phosphodiester bonds are so can't have alternate acceptor and donor sites.
  - donor and acceptor sites are mediated by splicing enhancers or splicing inhibitors.
  - Have special proteins that either direct splicing machinery to a different branch site or some factor sits either on the branch site or on an intron/exon junction so it cannot be used so a different one has to be used.
  - The consequence of alternative splicing either are degenerate diversity, meaning you get proteins that have a slightly different set of exons. Usually, most of the exons are shared. Then you can produce a unique exon or exon combination. Or can produce a non-functional product
- Fig 26.21 Alternative splicing involves differential use of splice junction
  - specific exons may be excluded or included in the RNA product by using or failing to use a pair of splicing junctions