Differences between RNA and DNA
1. Contains a 2’ OH rather than a H (Gives RNA unique structural properties)
2. Contains Uracil instead of Thymine
3. Forms extensive higher order structures

RNA can form extensive higher order structures

Bacterial Gene Expression
Only one polymerase transcribes all RNAs.
Only one cellular compartment.
The Ribosome can begin translating the mRNA as soon as it is transcribed.
In less than 5 minutes, transcription is completed, the mRNA is translated and degraded.

Gene Expression in Bacteria
DNA
$\downarrow$ Transcription
RNA
$\downarrow$ Translation
Protein
The RNA is co-linear with the DNA
3 Different RNA Polymerases in Eukaryotes

<table>
<thead>
<tr>
<th>RNA Polymerase</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>rRNA</td>
</tr>
<tr>
<td>II</td>
<td>heterogeneous nuclear (hnRNA) and mRNA</td>
</tr>
<tr>
<td>III</td>
<td>tRNA</td>
</tr>
</tbody>
</table>

Pre-mRNA is Spliced to form mRNA

Splicing: The process in which segments of the RNA (introns) are excised from the RNA molecule to form a contiguous mRNA.

Intron: Intervening sequences that are removed from the pre-mRNA.

Exons: Are the regions retained in splicing and correspond to the coding sequences of the mRNA.

Exon 1  Intron  Exon 2

Splicing

Discovery of Splicing

- RNA in the nucleus was longer than RNA in the cytoplasm.
- But knew that the cytoplasm RNA was derived from the nuclear RNA since was capped and polyadenylated.
- How/why does the RNA get smaller?

Human genes contain multiple exons

On average, human genes contain 8 exons.

Specific Examples:

- Factor VIII gene
- β-globin gene
Splicing Proceeds Through a Lariat Intermediate

How do we know what steps are involved in the removal of introns?

1. Development of an in vitro splicing assay enabled identification of the splicing pathway. The first step involves cleavage of exon 1, releasing the intron and exon 2. The intron contains a lariat structure.

2. The second step involves joining of exons 1 and 2 and the removal of the intron.

How does the cell know what regions to remove and join?

1. There are short conserved sequences at the exon-intron junctions to designate the splice sites.

2. Specific trans factors called small nuclear ribonucleoprotein particles (snRNPs, “snurps”) designate splice sites. Sequences that designate splice sites:

   1. Consensus sequence at the 5’ splice site
   2. Consensus sequence at the 3’ splice site
   3. The Branch A site

Splicing occurs within a large 60S Spliceosomal Complex

Specific Complexes recognize and designate the splice sites

A class of small U-rich RNAs (snRNA) form specific RNA-Protein complexes termed small nuclear ribonucleoproteins (snRNP) which is pronounced “snurps”.

There are 5 snRNPs involved in splicing:

- U1 snRNP
- U2 snRNP
- U4 snRNP
- U5 snRNP
- U6 snRNP

Collectively they form the core of the splicing machinery called the Spliceosome.
How do the snRNPs designate the splice sites?

By RNA-RNA interactions:
more precisely: snRNA-pre-mRNA Interactions

U4-U6 Interaction

U4 is thought to keep U6 in an inactive form until it is within the spliceosome

snRNA-pre-mRNA Interactions in Splicing

The Catalytic core of the Spliceosome??

Pathway of RNA Splicing

Figure 14.29

1. U1 snRNP designates the 5' splice site
2. Additional snRNP are recruited and
3. Additional snRNPs are recruited and

Proteins other than snRNP’s are involved in splicing

The major class of proteins other than the snRNP are the SR proteins
SR proteins are RNA-binding proteins that contain a stretch of Serine/Arginine repeats. The function of which is most likely in protein-protein interactions

SC35 was initially found to be important for spliceosomal commitment
Alternative Splicing
Can generate multiple proteins from the same primary transcript

Sex Determination in Drosophila Involves a Cascade of Alternative Splicing
Sex in Drosophila is determined by the ratio of X chromosome to a set of autosomal (A) chromosomes
XX AA would have an X:A ratio of 1 and be a Female
XY AA would have an X:A ratio of 0.5 and be a Male

The key regulator is the Sex lethal (Sxl) protein
Sxl is an RNA binding protein that binds to the 3’ splice site and represses splicing of that intron.
- Sxl autoregulates its own pre-mRNA splicing by preventing inclusion of exon 3 in females
- Sxl also regulates Tra pre-mRNA splicing to ensure a female specific splicing pattern
- The Tra protein is a positive regulator of Double-sex splicing

Why have introns?
- Could have facilitated the emergence of new proteins by exon shuffling.
- This would result in the combination of preexisting protein domains (single or several exons) into other genes.
- If the addition of a particular exon is not beneficial or is deleterious, it could simply be spliced out and the original gene is not altered.

Comparison of Group II and Nuclear mRNA splicing
They are almost identical except for the following several changes:

Group II
- Splicing can occur in the absence of proteins
- Does not require ATP
- High conservation of primary, secondary and tertiary structure

Nuclear mRNA Splicing
- Requires many proteins (> 10)
- Depends on ATP
- Minimal primary sequence conservation involved
**Fundamental Dilemma With the Current Central Dogma of Gene Expression in Explaining the Origin of Life**

DNA → RNA → Protein

In the Earliest Cell:

How was DNA replicated without Protein and RNA?

How was Protein made without DNA to encode it?

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**Role of RNA in the Origin of Life**

RNA World Hypothesis

RNA stored both genetic information and catalyzed the chemical reactions in primitive cells.

Enclosed by lipid bilayer

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**Why Do We Think RNA Was The First Biomolecule Able To Sustain Life?**

DNA is inert and is acted upon for replication and transcription

Protein is not known to self replicate

But RNA has many properties that fulfill many of the requirements:

1. RNA can be the genetic material
   - Many viruses still have an RNA genome

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Why Do We Think RNA Was The First Biomolecule Able To Sustain Life?

...But RNA has properties that fulfill many of the requirements:

2. RNA has catalytic activity and can act like a protein
   a. RNA was demonstrated to self-splice in 1982

3. RNA can self replicate

4. RNA is the essential component for translation
   - rRNA and peptide bond formation
   - tRNA
   - mRNA

5. Deoxyribose is derived from ribose, implying that RNA predated DNA

Potential Sequence of Events Leading to the Current Dogma