RNA processing

Proks vs. Euks
In proks, transcription & translation coupled
In euks, processes are temporally & spatially separated so more control
mRNA processing in eukaryotes:
- 5’-capping
- 3’-endonuclease cleavage and polyadenylation
- RNA splicing & editing

Diagram of the process:
- Transcription and 5’ capping
- Completion of primary transcript
- Cleavage, polyadenylation, and splicing
- Mature mRNA
RNA processing

mRNA processing:
5’-capping

(a)
5' End of RNA with triphosphate group

\[ \gamma \beta \alpha \]
\[ pppNp \]

phosphohydrolase
\[ \rightarrow P_i \]

ppNp
\[ \leftrightarrow \]
\[ \alpha \beta \gamma \]
\[ Gppp \]

GTP

guanylyltransferase
\[ \rightarrow PP_i \]

GpppNp

guanine-7-methyltransferase
\[ \rightarrow \]
\[ \text{adoMet} \]

m\(^7\)GpppNp

2'-O-methyltransferase
\[ \rightarrow \]
\[ \text{adoMet} \]

\[ \text{m}^7\text{GpppmNp} \]

5' End of RNA with cap

Figure 26-13b
Lehninger Principles of Biochemistry, Fifth Edition
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Figure 26-13c
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RNA processing

mRNA processing:
3’-endonuclease cleavage & polyadenylation
mRNA processing:
RNA splicing & editing
RNA processing

mRNA processing:
RNA splicing & editing
Mainly in eukaryotic organisms, highly conserved from yeast to human

Spliceosome is an RNA-protein complex; RNA proposed to be catalytic

**Figure 26-17b**
*Lehninger Principles of Biochemistry, Fifth Edition*  
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**Figure 26-17c**
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RNA processing

Alternative splicing
RNA processing

Alternative splicing
RNA Processing
Naturally occurring catalysts
Catalytic RNA = Ribozyme

- RNA cleavage
  - glmS ribozyme - also a RIBOSWITCH!! (crystal structure)
  - hammerhead ribozyme (crystal structure)
  - hairpin ribozyme (crystal structure)
  - Varkud satellite (VS) ribozyme (partial NMR structure)
  - hepatitis delta virus (HDV) ribozyme (crystal structure)
  - M1 RNA (RNase P) (partial crystal structure)

- RNA splicing
  - group I introns (crystal structure)
  - group II introns (partial crystal structure)
  - *** U2-U6 snRNA (spliceosome) (partial NMR structure) ***

- Peptide bond formation
  - ribosome (crystal structure)
RNA processing

RNA catalyzed splicing (*ribozymes*)

Group I intron – found in fungi, algae, plants, bacteria

Group I introns found in nuclear, mitochondrial and chloroplast genes of rRNAs, tRNAs and mRNAs
Group I Intron crystal structure
RNA processing

RNA catalyzed self-cleavage (*ribozymes*)

Hammerhead ribozyme (found in a virusoids, part of small RNA genome)
RNA processing

Riboswitches

Riboswitches contain an aptamer (RNA binding) domain & expression platform
Riboswitches


Newly discovered riboswitches glycine, pre-Queuosine, 2’-deoxyguanosine, cyclic dimeric GMP
• Transcription termination and anti-termination
• Translation initiation: RBS accessibility
• RNA processing: Splicing or degradation

• Recognizes ligand phosphate through metal ion-mediated backbone and nucleobase contacts
• Riboswitch regulation of gene expression is perturbed by the antibiotic PTPP
• Riboswitch agonists or antagonists: novel antibiotics?

**Tempting Targets**

Many bacteria, including the human pathogens listed here, employ riboswitches to control the activity of their own genes. Agents that trigger those riboswitches might therefore serve as new antibiotics, particularly if the drugs disrupt the function of genes essential to an organism’s virulence or survival. The number of riboswitch classes found in each organism and the number of genes known to be regulated by riboswitches are shown below. Asterisks indicate that at least one vital gene is regulated by a riboswitch.

<table>
<thead>
<tr>
<th>Human Bacterial Pathogen</th>
<th>Riboswitch Classes</th>
<th>Genes Regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><em>Bacillus anthracis</em></td>
<td>9</td>
<td>82</td>
</tr>
<tr>
<td><em>Brucella melitensis</em></td>
<td>5</td>
<td>21*</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>4</td>
<td>15*</td>
</tr>
<tr>
<td><em>Francisella tularensis</em></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><em>Hemophilus influenzae</em></td>
<td>5</td>
<td>15*</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>9</td>
<td>49</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>3</td>
<td>34*</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>8</td>
<td>30*</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td><em>Yersinia pestis</em></td>
<td>3</td>
<td>11</td>
</tr>
</tbody>
</table>

Glucosamine 6-phosphate riboswitch


A conserved RNA structure precedes the *glmS* gene in Gram-positive organisms

**RNA Interference (RNAi)**

- RNAi is the process whereby double-stranded RNA (dsRNA) induces homology-dependent degradation of cognate RNA (*i.e.* gene silencing)
- RNAi is highly conserved among eukaryotes (fungi, protozoans, plants, nematodes, invertebrates, mammals)

**Biological significance of RNAi**

**Cellular immune response to viruses**

In certain organism (especially plants), RNAi serves as a first line of defense against viral infection, as viral replication typically includes dsRNA species

**Genetic stability**

RNAi repress the mobility of transposable genetic elements in *C. elegans* and *S. pombe* which requires the formation of dsRNA

**Organismal development and germline fate**

Developmental processes are affected by endogenous non-coding RNAs that function through the RNAi pathway (microRNAs)