RNA Structure and Function

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Living cells have developed an extremely accurate and dependable method of information storage. This system employs two types of nucleic acid polymers to store information: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Both of these nucleic acid polymers are capable of base pairing with each other and with themselves. When the nucleic acid polymers base pair with a complimentary strand of the same type (either DNA-DNA or RNA-RNA) the strand is called a homoduplex. Similarly, when DNA and RNA compliments bind, it forms a heteroduplex. The stability of the heteroduplexes and homoduplex are identical. Although both contain the word "acid", neither has a single acidic property and both perform basic chemistry.

Central Dogma

The relationship of DNA and RNA is best explained in the Central Dogma proposed by Francis Crick in 1958. A diagram of the Central Dogma is shown below. The location is shown in red and the job it does is shown in blue.

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DNA is considered the information storage mechanism of the cell and can undergo replication during cell division to produce more copies. While DNA contains the genetic code of our proteins it does not directly encode for proteins. To be translated to proteins, DNA would need to leave the nucleus and travel through the cytoplasm to get to the ribosomes. This is a terrible idea because DNA would get cut to pieces by cellular nucleases in the cytoplasm. Thus RNA acts as the intermediate molecule which will act as a messenger (mRNA) to prevent DNA from having to leave the nucleus. DNA can form RNA copies of itself by undergoing transcription. Once transcribed, the RNA will leave the nucleus and travel through the cytoplasm (where it exists most of the time) to the ribosome, where it is translated into proteins.

Since the RNA stays in the cytoplasm most of the time, it is the molecule that gets cut into pieces by nucleases. This makes sense for several reasons. RNA has a half-life of only hours. If RNA were to last forever, it would continue to make an infinite amount of proteins in your body. Think of it this way, if RNA were to last forever, you wouldn't. DNA on the other hand, needs to last forever so it should not leave the safety of the cell's nucleus.

Difference between DNA and RNA

While DNA and RNA look very similar, there are two critically important differences between them. The key difference is that there is a 2' hydroxyl (OH) group in RNA. This hydroxyl is completely absent from DNA. The benefit of this hydroxyl group is that RNA can undergo more hydrogen bonding than DNA. In other words, it can fold into interesting conformations and 3D structures that DNA is not capable of. One example of such RNA folding that we have seen so far is tRNA. Another benefit of the hydroxyl group is that it makes RNA more reactive towards hydrolysis. This will further be explained in the section Ribose Reactivity.

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The second difference between RNA and DNA is that RNA uses uracil instead of thymine as the complementary base to adenine. The odd thing is that thymine and uracil are almost identical. The only difference is that thymine has a methyl group whereas uracil doesn't. This additional methyl group does not

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Here are examples of base paring with RNA. Note the Hydroxyl on the 2'-carbon of the Ribose on the RNA base pairs pictured below.

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Note the lack of a methyl group on the 5-carbon of the Uracil below and how it does not affect hydrogen bonding between itself and adenosine.

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So why can't DNA just use uracil instead of thymine? Cytosine, a purine used in both DNA and RNA can undergo hydrolysis to change into uracil, a purine found ONLY in RNA). Even though the hydrolysis of cytosine to uracil can take several years to successfully complete, the average human being capable of reading this wiki has been alive for several years and some of the cytosines in his or her body will have already been hydrolyzed into uracils. If DNA used uracil, this damage would not be recognized and the hydrolyzed cytosine (now a uracil) would base pair with an adenine instead of a guanine (the cytosine base pair) during replication, and the mutation would be propagated. Because DNA uses thymine, any uracil present in DNA will be recognized as a damaged cytosine and can be fixed. When cytosine hydrolyzes to uracil, base pairing is interrupted, disrupting DNA replication and translation. To prevent this disruption, our bodies have developed an enzyme that runs down DNA strands looking for rogue uracils by recognizing them as NOT having the methyl group that thymine would have. They are then cut out and replaced with cytosines.

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(note: While RNA also contains cytosines that can also undergo hydrolysis to form uracils, this never occurs because RNA has a half-life of only a couple of hours. The hydrolysis of cytosine to uracil takes several years. RNA is quickly destroyed by RNA nucleases, so long term stability is not needed. If the hydrolysis of cytosine were to occur, RNA is not copied, so errors are not propagated.)

RNA Reactivity

As discussed earlier, the 2'-hydroxy group on RNA greatly increases its susceptibility to hydrolysis. DNA is impervious to attack by a base because the oxyanions of phosphorous will repel the hydroxide nucleophile.

This is one of the central reasons for which DNA is used in place of RNA for our genetic code. This can be seen as an evolutionary advantage as the genes will be more reliably replicated, and less likely to be denatured, or mutated by the increasing susceptibility by hydrolysis. RNA is reliable enough to perform the other steps in translation and transcription. There would be more chance of mutation or damage to the RNA being translated, but this is not a big issue evolutionarily because those mistakes will be short lived and won't be detrimental to the fitness of the organism. This is one aspect of the RNA World Hypothesis.

DNA Hydrolysis

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However, RNA will hydrolyze completely. This is because in RNA, the 2'-hydroxy group provides a "back door" for the nucleophile to attack. The hydroxide will deprotonate H from the 2'-OH group and the electrons from the O-H bond will flow down to phosphrous and perform an Sn2 substitution, ejecting the oxygen containing the 2nd ribose as a leaving group. Now there is a ribose intermediate in which two cis oxygens are bonded to the phosphate. This is also favorable because an intramolecular reaction results in 5-membered ring formation. From this point, water can attack from either side in another Sn2 substitution leading to either 3'-NMP or 2'NMP. The yield is 1:1.

RNA Hydrolysis

Use the color coding to follow the side of attack and respective products in the mechanism below. Also, note

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Although this mechanism (as illustrated below) is the least common it is in fact possible, allowing for the release of the phosphate from the top RNA molecule. It is the least common pathway because it is kinetically difficult for the hydroxide, which is not pre-positioned, to attack the phosphate through the electron rich rich oxygen clouds. The phosphate is a weak nucleophile and the phosphate oxygen bond has very little double bond character. The release of the phosphate from the top RNA molecule would not occur if you attempted to attack from the other side as normal, shown in the previous figure. The 2'-hydroxyl will not directly break the 3' oxygen-phosphate bond because the electrons from the deprotonated hydroxide would have to travel down to the sigma star orbital of that bond, and they cannot reach this sigma star orbital (shown below). The impossibility of that reaction is good for RNA because it keeps the backbone uniform instead of flopping back and forth.

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In the observered reaction, however, it is easy to see the P-O sigma star orbital is accessible to the oxygen's electrons.

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RNA Nucleases

We don't have hydroxide ions in our cells, only water, which is too slow at cleaving RNA. Therefore we need an enzyme to help with this process. RNA nucleases' sole job in the cytoplasm is to find RNA and cut them up into RNA monomers. The mechanism of RNA nuclease is very similar to the previous one. Inside each nuclease active site are two histidines (similar to the active site of *phosphoglycerate mutase* in glycolysis), one on each side of the ribose phosphate backbone. One histidine will deprotonate the 2'-hydroxy group on the ribose. The electrons from this bond attack the phosphate in an Sn2 reaction, ejecting the 5'-oxygen as a leaving group and cleaving the backbone. The other histidine residue on the opposite side deprotonates a bound water (similar to the active site of aspartyl proteases) which then attacks the phosphate in another Sn2 reaction. Different RNA nucleases produce either 2'NMP or 3'NMP depending on which side the bound water is on.

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As mentioned before, RNA, unlike DNA, cannot last forever because as a result its messages would be created repeatedly. This is unwanted because RNA would endlessly transcribe DNA, which would result in a deadly abundance of proteins.To solve this problem, we have enzymes that catalyze RNA hydrolysis: RNA nucleases. These enzymes speed up the process of RNA degradation.

As shown in the mechanism below, 2 Histidines and water are needed. The neutral histidine activates the 2' hydroxyl to attack the phosphate. The positively charged histidine enables the other hydroxyl to be a good leaving group. Are you wondering why exactly we use histidine? Well, in order for this mechanism to perform successfully, you need an amino acid that is neutral. A neutral characteristic allows it to function as an acid and a base. An amino acid with stronger or weaker acid/base quality is less effective in activating the hydroxyls. With a pka of 7 (pka describes molecule), Histidine is the perfect candidate for a RNA nuclease.

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In addition to preventing persistence of message processing, RNA nucleases serve two other purposes. Like DNA nucleases, they break down exogenous RNA and recycle RNA from cells that have died; a vital process for a cell, which must absolutely save and recycle as much energy as possible.

Thymidylate Synthase

The difference between uracil and thymine is only a methyl group yet the mechanism for this reaction is lot more complicated. Instead of using the cofactor SAM to add the methyl group our body uses N5, N10

methylene-THF.

1) Electrons on nitrogen will collapse to form a π bond, breaking open the ring. This gives you an imminium.

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2) An enzymatic base will remove a proton and electrons on oxygen will collapse to form a carbonyl and kick the π bond over to attack CH2 on the cofactor, forming a new carbon-carbon bond. Your cofactor is now connected to your base! The π bond from CH2 will flow back to nitrogen to stabilize its positive charge. This type of aldol addition is a Mannich Reaction because it is a nitrogen derivative being attacked.

3) Enzymatic bases are used to tautomerize the molecule to make it easier to remove the cofactor.

4) Now it's time to remove the THF cofactor via conjugate elimination. An enzymatic base will deprotonate the hydrogen, the electrons will collapse to form carbonyl and kick the π bond over to eject the nitrogen leaving group.

5) We are not done with the cofactor. Our base needs a CH3 not a CH2. In a mechanism similar to NADH, the cofactor transfers a hydride to the substrate via a conjugate addition.

6) Now we must get rid of the cysteine. An enzymatic base again deprotonates the hydrogen and electrons collapse to form a carbonyl. It kicks the π bond over and ejects the cysteine side-chain which picks up a proton on its way out.

7) The base has a ribose ring attached to it (Before, it was represented by the letter R to save space), and we can phosphorylate the base by gamma addition of a phosphate from ATP.

8) The new molecule is called nucleotide monophosphate or NMP. However, there is a 2'-OH that is not a part of DNA. In order to produce thymine, the 2' hydroxyl group must be ripped off. This step is called dehydroxylation and will be covered below.

The cell goes through all this trouble to make thymine out of uracil so that the repair enzyme that scans our DNA for uracil can distinguish between the two bases. If there was no distinction between thymine and uracil, the repair enzyme would rip off the thymine and replace a cytosine in its place. As mentioned earlier, this can be dangerous during replication or translation.

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Thymidylate Synthase Inhibitors

The purpose of inhibiting Thymidylate Synthase is to inhibit DNA replication. This is very useful if we want to prevent cancer cells from duplicating. While thymidylate synthase will also affect normal cells, cancers replicate more rapidly and thus will be hurt the most.

There are two molecules that can be used to inhibit thymidylate synthase. One is 5-fluorouridine. It is an analog of thymine but has a fluorine where a hydrogen should have been. This molecule inhibits tautomerization (the third step of thymidylate synthase) thus locking the cofactor onto the 5-fluorouridine. It is an irreversible inhibition because 5-fluoridine is still attached to a cysteine.

Another molecule that inhibits thymidylate synthase is trifluorothymidine. Its mechanism is identical to DFMO (a PLP inhibitor). An enzymatic base removes a proton from the hydroxyl group, the carbonyl collapses, and kicks off a fluoride anion. A nucleophile then does a conjugate addition onto the substrate. Finally, another enzymatic base will do another conjugate elimination of a second fluorine anion. Now the molecule is stuck because there is nothing left to eliminate. This inhibition is also irreversible because the molecule is now doubly linked (crosslinked) to the enzyme.

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Ribonucleotide Reductase

On the second to last step of Thymidilate Synthase, the 2' hydroxyl is ripped off of NMP to produce DNA. The

NMP is reduced to dNMP and a disulfide bond is oxidized into two SH cysteine amino acids. Producing FAD via oxidation of FADH2.

Drill Problems

D25-1. If one hydrolyzes the isotopically-labelled RNA molecule shown below, what products are formed? Show clearly the stereochemistry at phosphorus for all proposed products.
<script>//<![CDATA[cd_includeWrapperFile('/download/attachments/4653090/'); //]]> <script>//<![CDATA[cd_insertObjectStr("<EMBED src='/download/attachments/10223653/$body' width='600' height='300' viewOnly='true' align='left' border='1' type='chemical/x-cdx' name='cdl'>"); //]]>

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D25-2. Provide a mechanism for the hydrolysis of cytosine to uracil. You may use either acid or base catalysis.

D25-3. Draw all major products produced by the treatment of the methylated nucleotide below with NaOH. Describe how these products are made.

<script>//<![CDATA[cd_includeWrapperFile('/download/attachments/4653090/'); //]]> <script>//<![CDATA[cd_insertObjectStr("<EMBED src='/download/attachments/10223653/$body' width='600' height='300' viewOnly='true' align='left' border='1' type='chemical/x-cdx' name='cdl'>"); //]]>

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D25-4. Would you expect the rate of hydrolysis of RNA under acid catalysis to be faster, slower, or about the same as the rate under base catalysis. Why?

D25-5. Show how the outcome of using 18O-labeled hydroxide anion in an RNA hydrolysis mechanism would definitively demonstrate that the hydroxide does not directly attach the phosphorus backbone of RNA.

Answers

Drill25-1.mov	
Drill25-2.mov	Drill25-2.mp4
Drill25-2-2.mov	Drill25-2-2.mp4
Drill25-3.mov	Drill25-3.mp4

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