Alcohol dehydrogenase

Alcohol dehydrogenase; aldehyde reductase;ADH; alcohol dehydrogenase (NAD); aliphatic alcohol dehydrogenase; ethanol dehydrogenase; NAD-dependent alcohol dehydrogenase; NAD-specific aromatic alcohol dehydrogenase; NADH-alcohol dehydrogenase; NADH-aldehyde dehydrogenase; primary alcohol dehydrogenase; yeast alcohol dehydrogenase
Oxidoreductases; Acting on the CH-OH group of donors; With NAD+ or NADP+ as acceptor

Ethanol Metabolism

- Ethanol is;
  - Small 2-C molecule of alcohol, alcoholic hydroxy group
  - Soluble in aqueous, lipid media. Thus, free passage into bodily fluids
  - Metabolised by 3 mechanisms:
    - Alcohol dehydrogenase (ADH) – most important
    - Microsomal ethanol oxidizing system
    - Fatty acid ethyl ester synthase – non-oxidizing pathway
    - Catalase (less significant)

Ethanol Metabolism Contd…

- Converted to acetaldehyde by alcohol dehydrogenase (ADH);
  - ADH: homo- and heterodimers of α, β, γ sub-units
    - high ethanol oxidation activity
  - Acetaldehyde: highly unstable, toxic, destroy embryonic neural crest cells, birth defects, liver and kidney damage

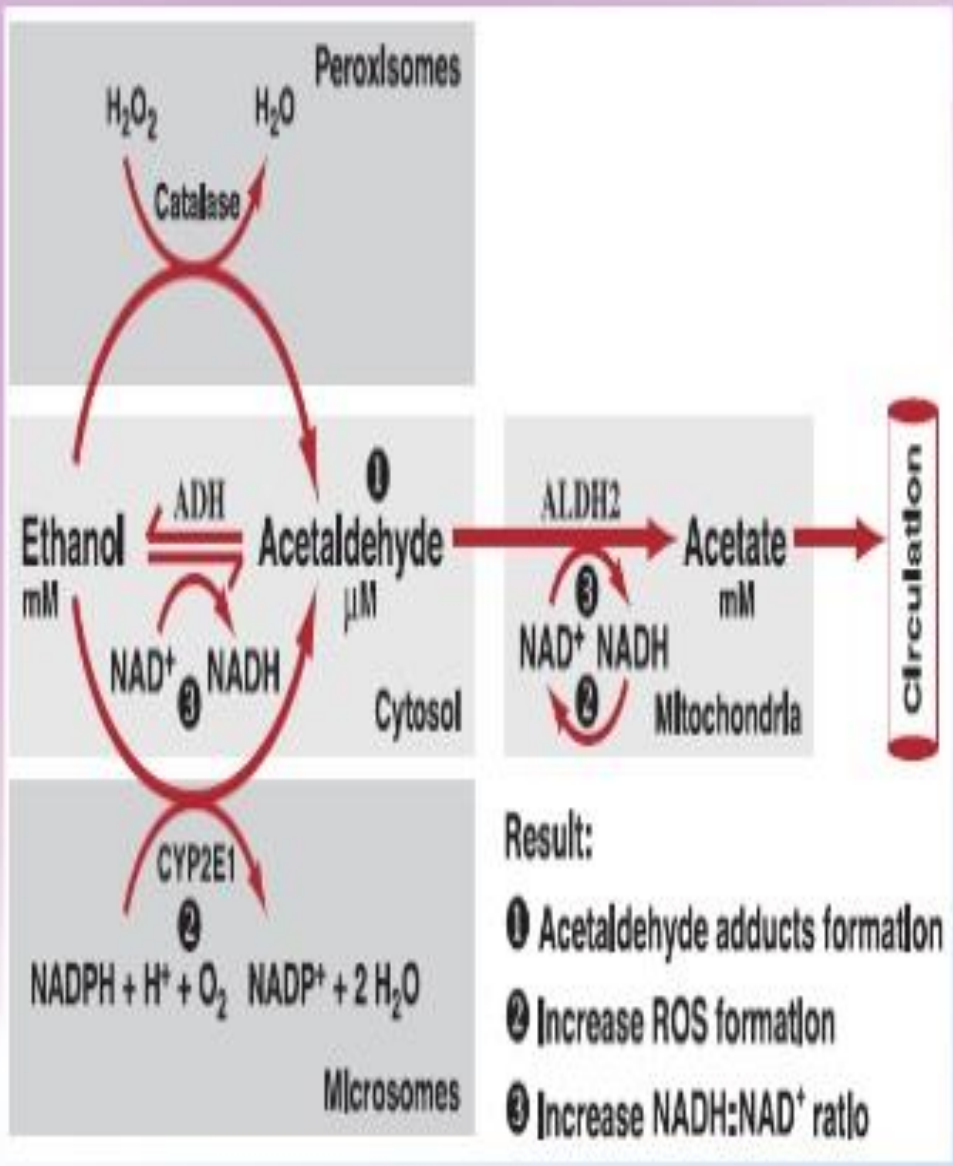


Figure 1: Oxidative pathways of alcohol metabolism: alcohol dehydrogenase (ADH), CYP2E1, catalase. Metabolism of alcohol. ADH, present in the fluid of the cell (i.e., cytosol), converts alcohol (i.e., ethanol) to acetaldehyde. This reaction involves an intermediate carrier of electrons, + nicotinamide adenine dinucleotide (NAD), which is reduced by two electrons to form NADH. Catalase, located in cell bodies called peroxisomes, requires hydrogen peroxide (H2O2) to oxidize alcohol. CYP2E1, present predominantly in the cell's microsomes. (1).

- Alcohol (ethanol or ethyl alcohol) is readily absorbed by the stomach & intestine.
- Only 1% of the alcohol consumed is excreted through lungs, urine & sweat.
- Major fraction of the alcohol is oxidized in liver.
- Alcohol gets oxidized in the liver by alcohol dehydrogenase to acetaldehyde.

Alcohol Dehydrogenase (ADH)

- It is an NAD^+ dependent cytoplasmic enzyme.
- It oxidizes ethanol to acetaldehyde.
- ADH is a dimer & has 6 isoenzymes.
- In some individuals the enzyme is mutated.
- In such individuals, alcohol metabolism is slower & even small quantity of alcohol may produce symptoms of intoxication.

Alcohol Metabolism

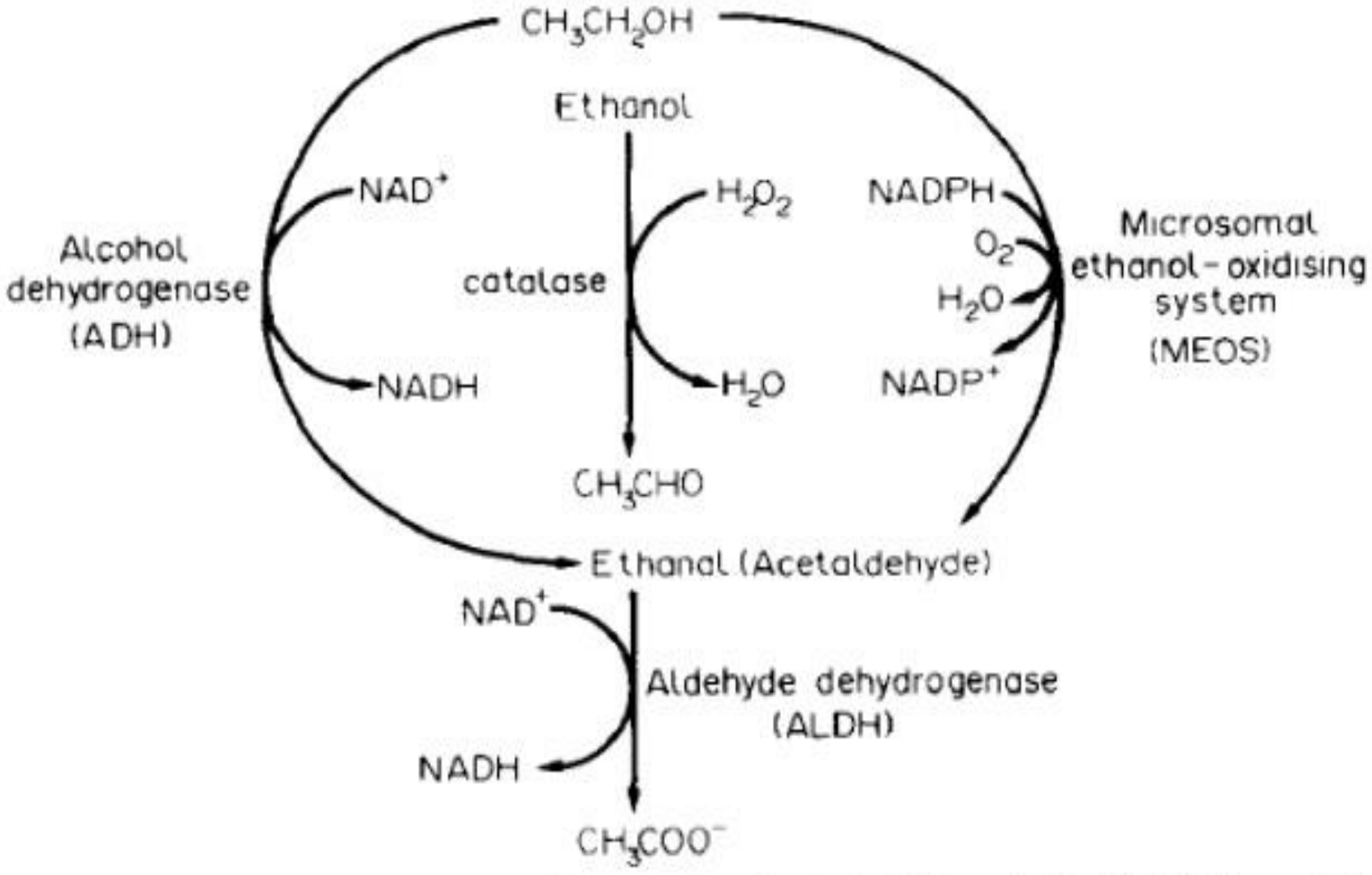
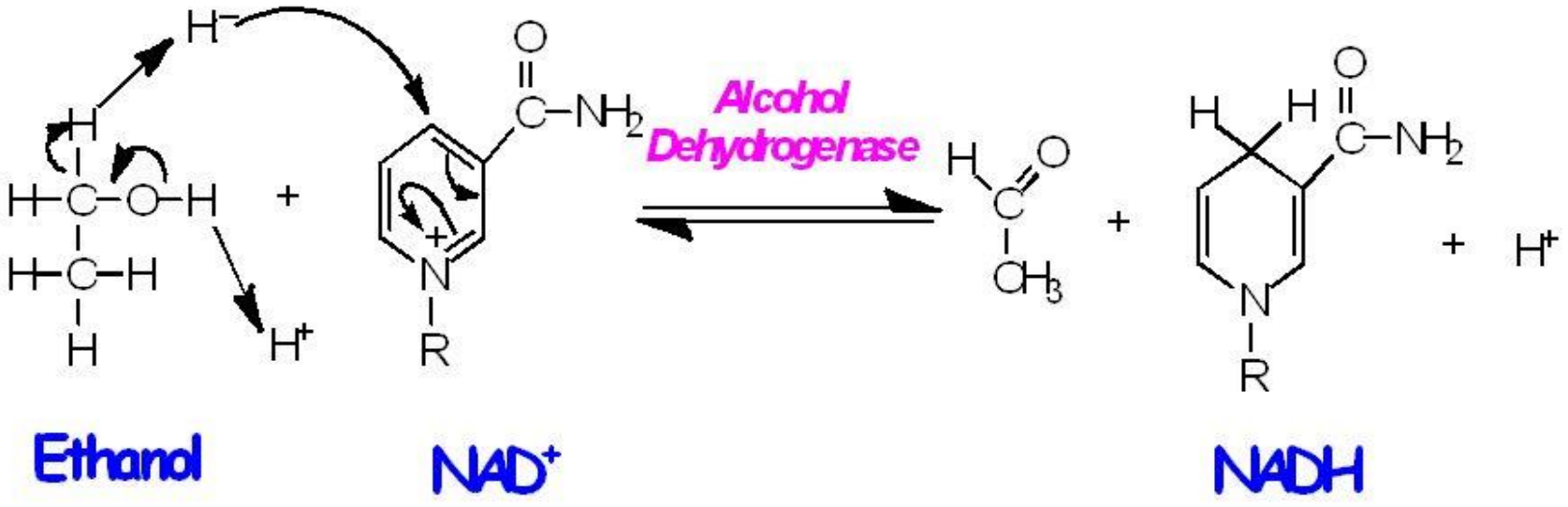


Figure 2: The Basic Pathways (2)

Alcohol Dehydrogenase

(Oxidation-Reduction Reaction)

Only one of the protons of EtOH is abstracted and is added to a specific side of NAD^+.



Mechanism of alcohol dehydrogenase. Note that the Zinc atom is coordinated in the active site by Cys-174, Cys-46 and His-67, however, these residues were left out of the mechanism to emphasize the active residues.

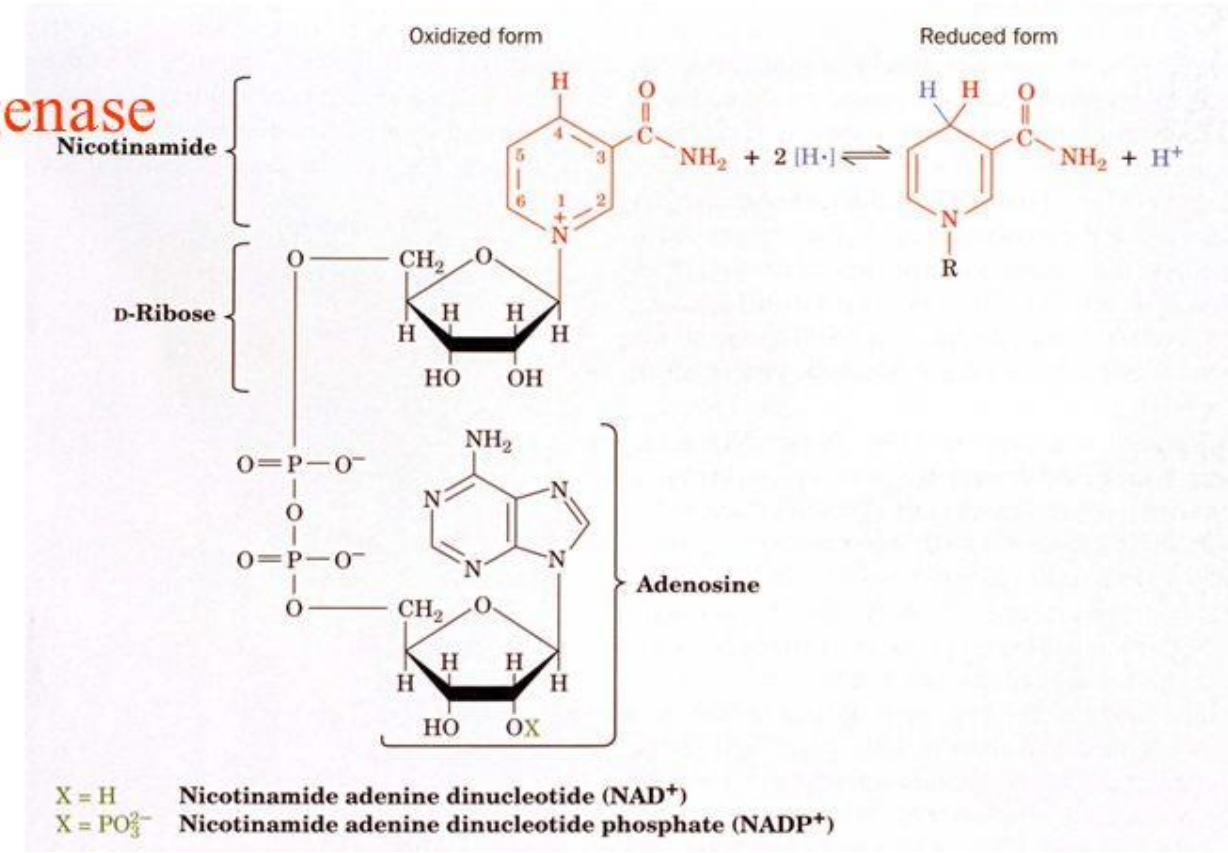
ADH is an oxidoreductase enzyme that oxidizes alcohol to acetaldehyde while subsequently reducing an NAD^+ cofactor to NADH. A Zn^{2+} atom is coordinated in the active site by Cys-174, Cys-46 and His-67 and functions to position the alcohol group of ethanol in the active site. Ser-48 and His-51 function similarly to a catalytic dyad, acting as a charge-relay network to help deprotonate the ethanol and activate it to be oxidized to the aldehyde. Before ethanol enters, a water molecule is initially positioned in the active site, but dissociates when the ethanol enters. At the end of the mechanism, water again enters the active site when the oxidized substrate—acetaldehyde—leaves

Alcohol dehydrogenase (ADH) is located in the cytosol of stomach and liver cells and functions as the main enzyme for alcohol metabolism . ADH has a low K_m and becomes saturated, reaching its $V_{max,}$ even at low concentrations of ethanol. Therefore, the enzyme appears to show zero-order kinetics because once the enzyme is saturated, the reaction rate is no longer dictated by the concentration of the ethanol

Enzymes are Stereospecific

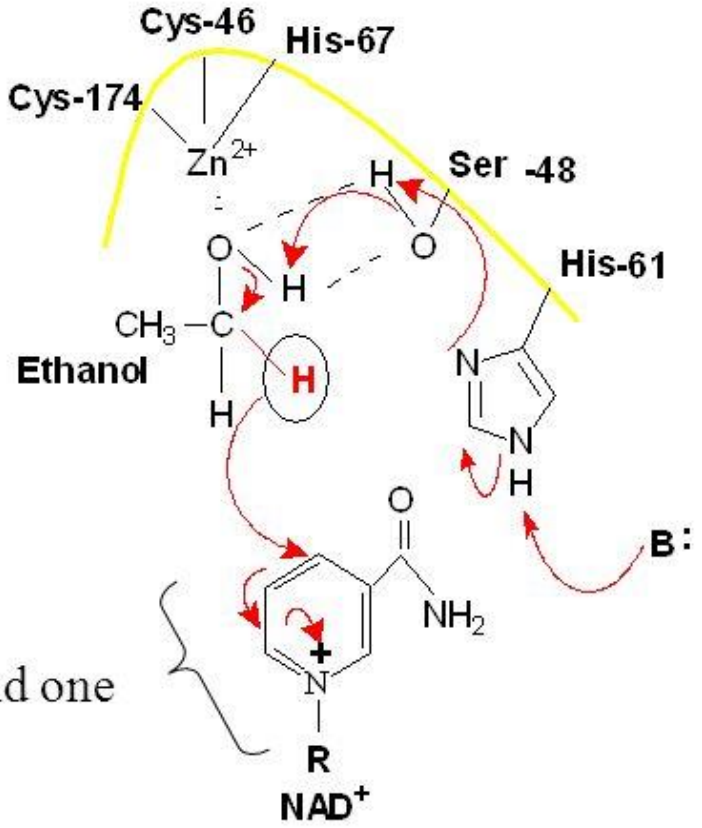
$$CH_3CH_2OH + NAD^+ \Leftrightarrow CH_3\overset{\overset{O}{\|}}{C}H + NADH + H^+$$

Alcohol dehydrogenase



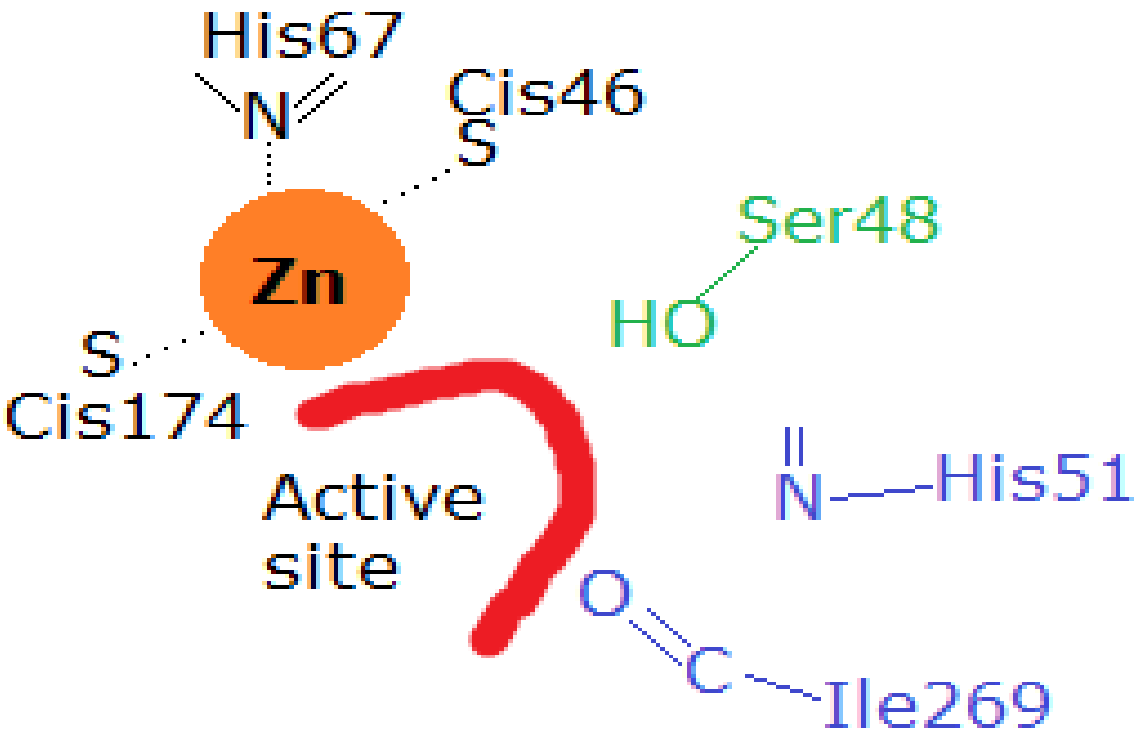
Mechanism of Alcohol Dehydrogenase

The Zn^{2+} increases the acidity of the alcohol, but is not involved in the redox reaction

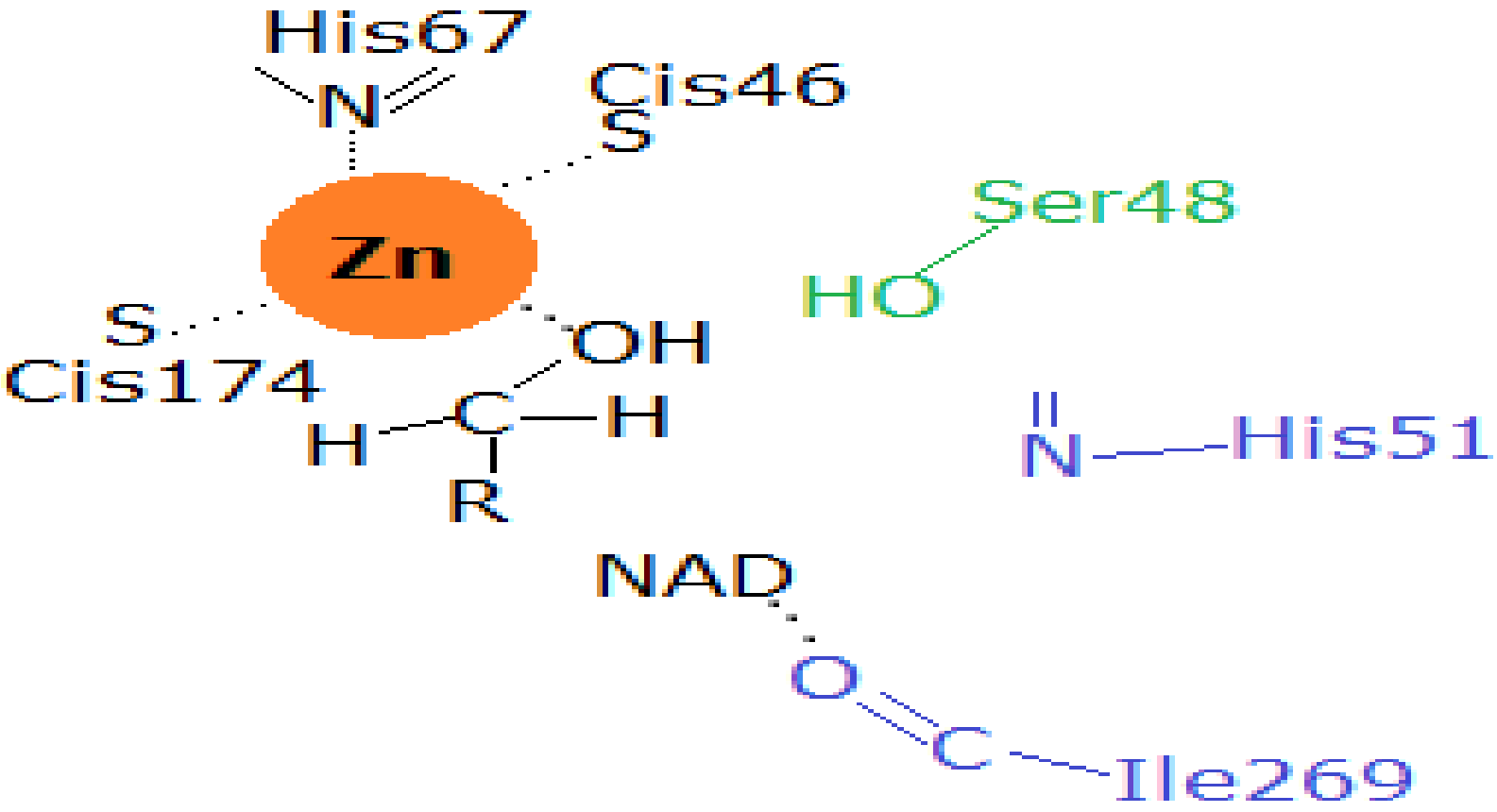


Electron sink (Stored 2 electrons and one H^+). Source & Where?

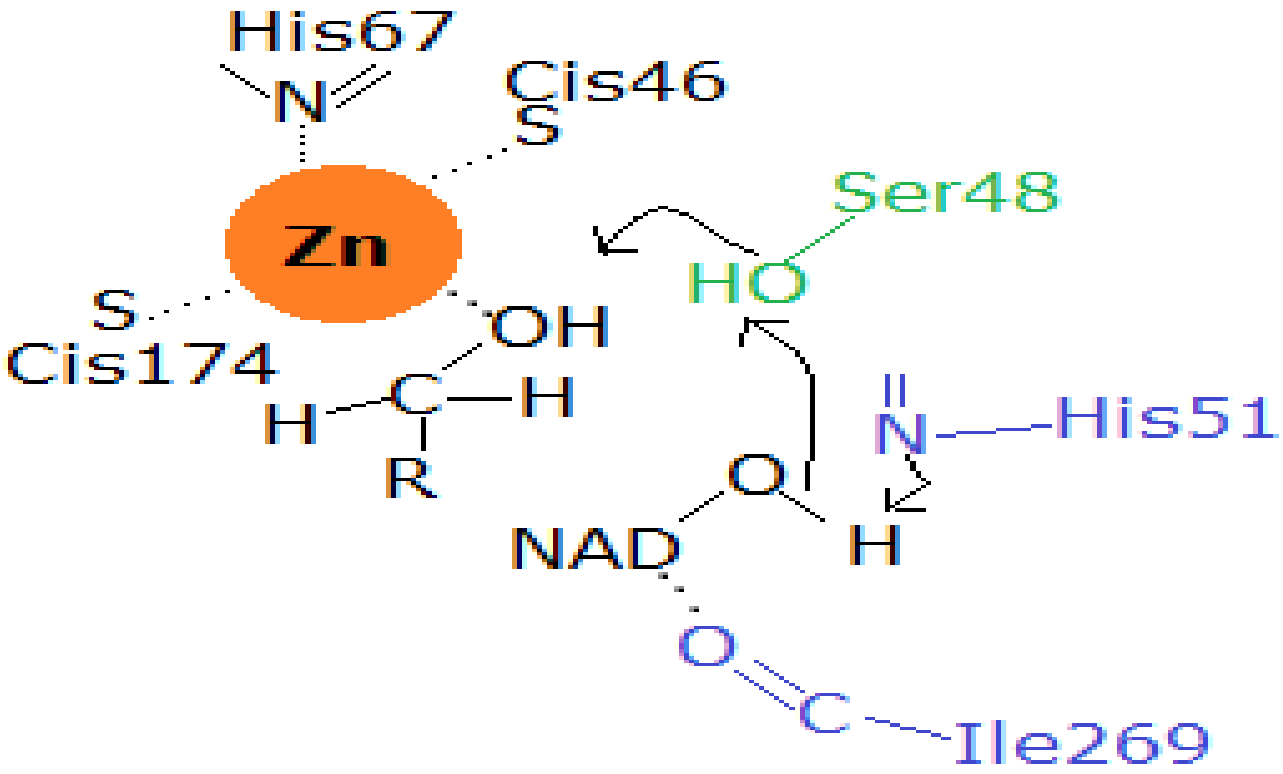
The active site of alcohol dehydrogenase includes a serine, a histidine, an isoleucine and a zinc stabilized by two cysteines, and a histidine.



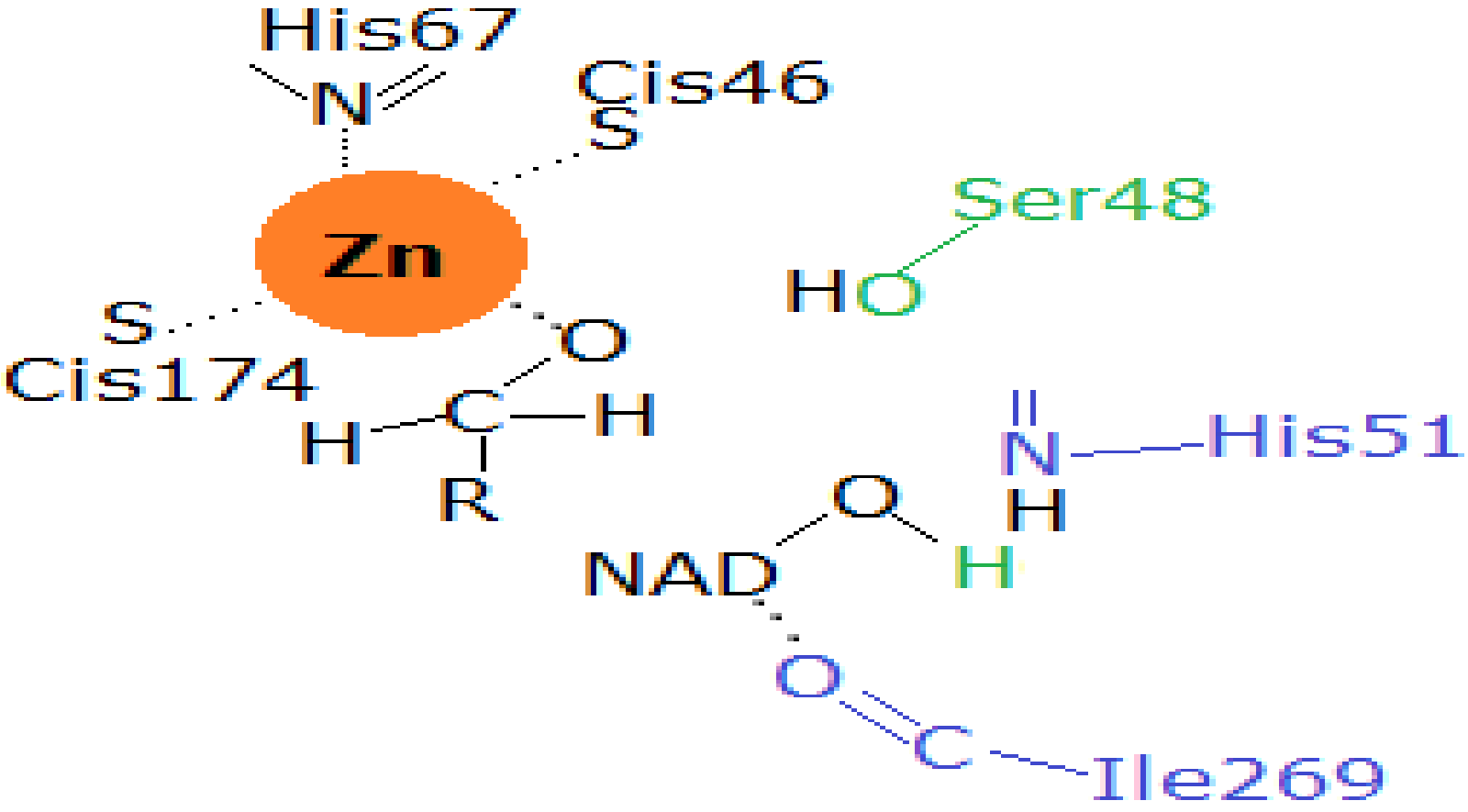
The active site includes a zinc, serine, hisitidine, isoleucine, and cysteine



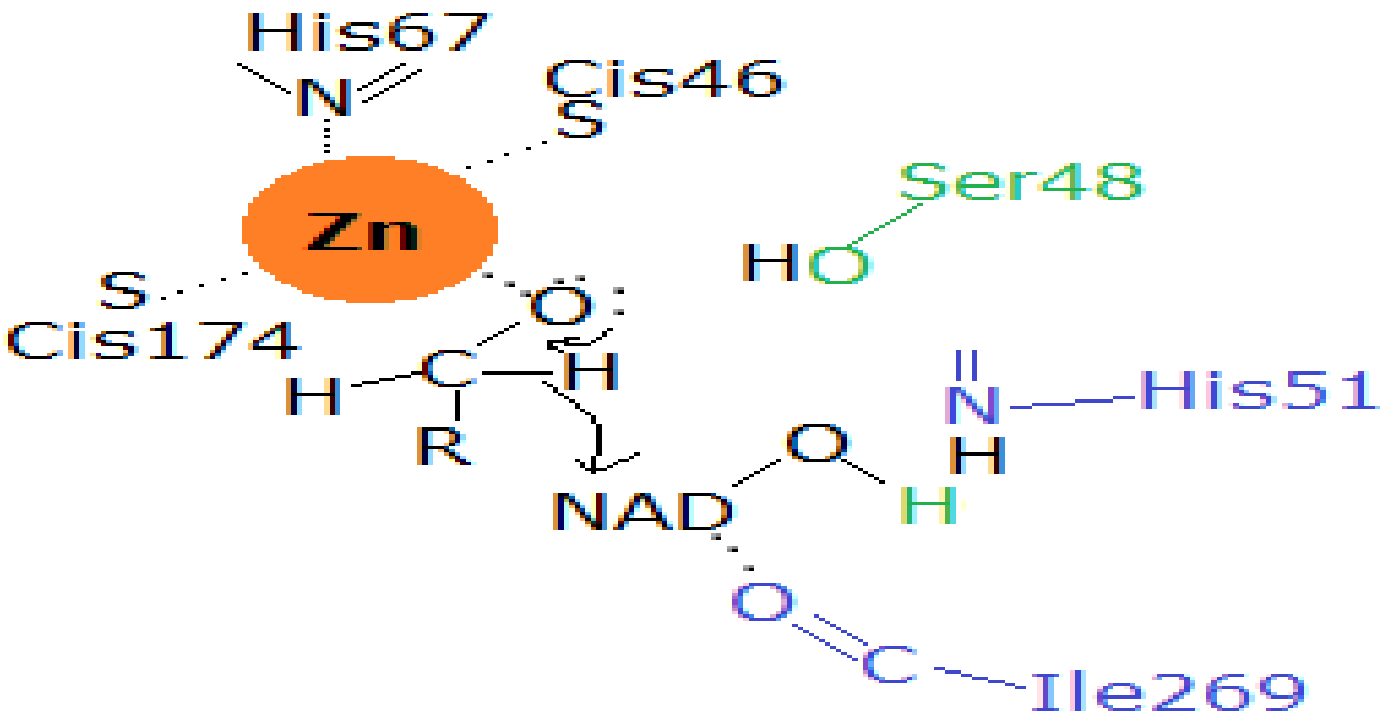
The alcohol and NAD are connected to the enzyme with the isoleucine and zinc



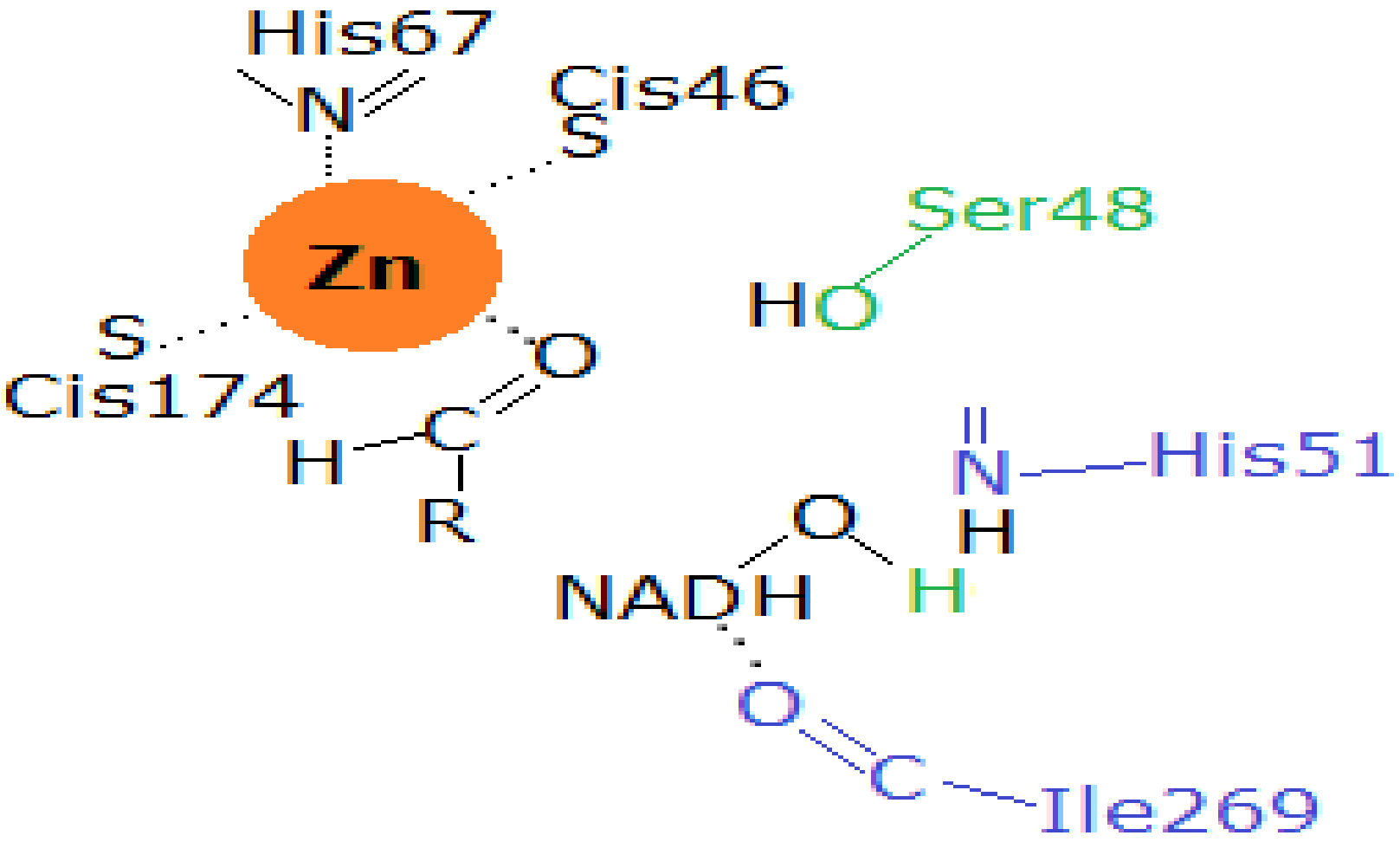
Histidine removes a hydrogen from NAD, which in turn removes a hydrogen from serine, which removes the hydrogen from the alcohol



The products of step 1 includes a NAD molecule and the alcohol without a hydrogen on the oxygen



In step 2 the hydrogen is removed from the carbon and put onto NAD, forming NADH, also the carbon oxygen double bond is formed.



We end up with an aldehyde and NADH

RIBOZYME

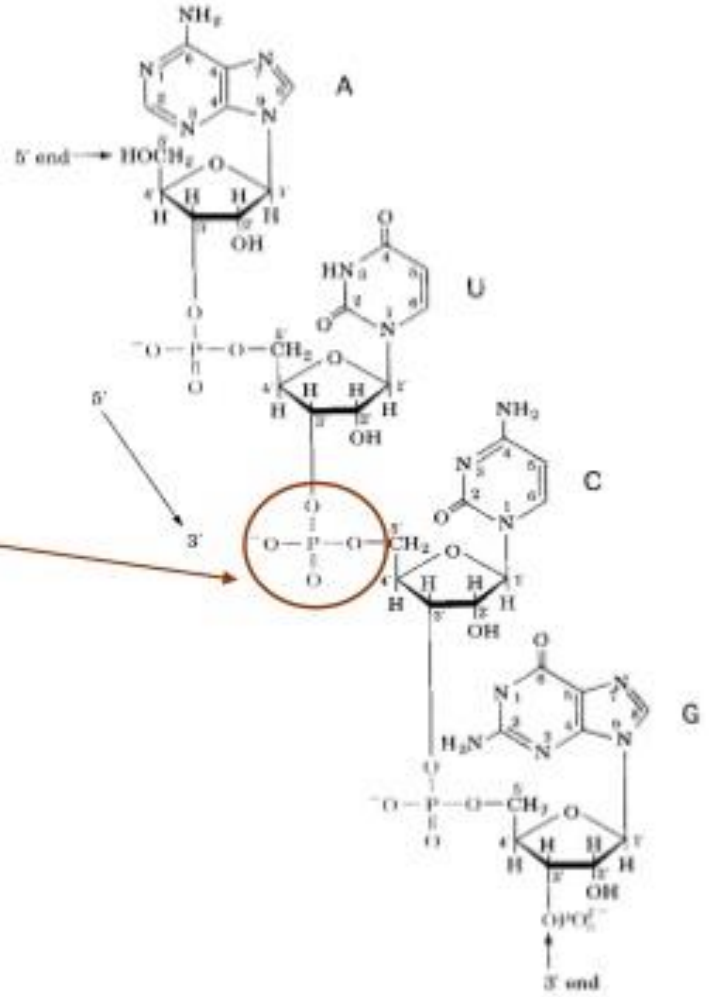
A ribozyme (Ribonucleic acid enzyme) is an RNA molecule that is capable of performing specific biochemical reactions similar to the action of protein enzymes

RIBOZYMES

- RIBOZYMES are *RNA* possessing *catalytic* activity.
- The first Ribozymes were discovered in the *1980*s by *Thomas R. Cech* and *Sidney altman*.
- The term "ribozyme" was first introduced by *kelly kurger* et al. in *1982*

RIBOZYME:

- RNA possessing catalytic activity
- Increases the rate and specificity of:
  - phosphodiester bond cleavage
  - peptide bond synthesis
- Widespread occurrence in nature – from viruses to humans



Characteristic features of RNA molecule are:

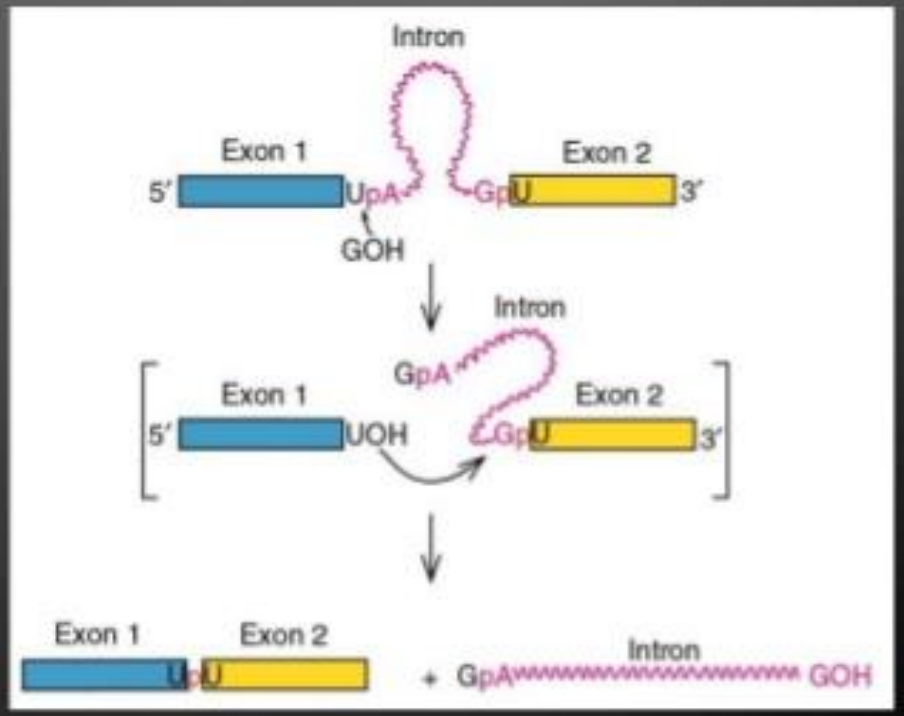
- An enzyme that uses RNA as a substrate
- AN RNA with enzymatic activity
- An enzyme that catalyzes the association between the large and small ribosomal subunits
- An enzyme that synthesizes RNA as part of the transcription process
- An enzyme that synthesizes RNA primers during DNA replication

Group I Intron Splicing :

- Group I intron ribozymes constitute one of the main classes of ribozymes.
- Found in bacteria, lower eukaryotes and higher plants.
- Group I introns are also found inserted into genes of a wide variety of bacteriophages of Gram-positive bacteria.
- However, their distribution in the phage of Gram-negative bacteria is mainly limited to the T4, T-even and T7-like like bacteriophages.

Mechanism:

The group I splicing reaction requires a guanine residue cofactor, the 3' OH group of guanosine is used as a nucleophile. The 3' OH group attacks the 5' phosphate of the intron and a new phosphordiester bond is formed. The 3' OH of the exon that is displaced now acts as the nucleophile in a similar reaction at the 3' end of the intron. So the intron is precisely excised and exons are joined together.

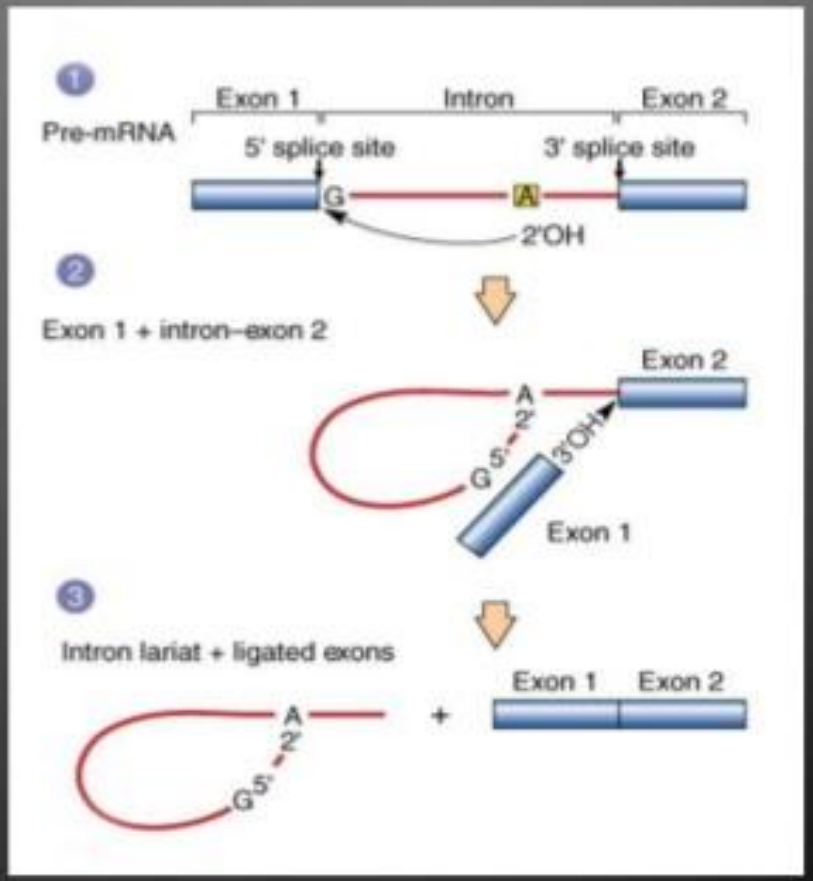


Group II Intron Splicing :

- Group II introns have been found in bacteria and in the mitochondrial and chloroplast genomes of fungi, plants, protists, and an annelid worm.

Mechanism:

The 2'OH of a specific adenosine acts as a nucleophile and attacks the 5' splice site creating a branched intron structure. The 3' OH of the 5' exon attacks the 3' splice site, ligating the exons and releasing the intron as a lariat structure.

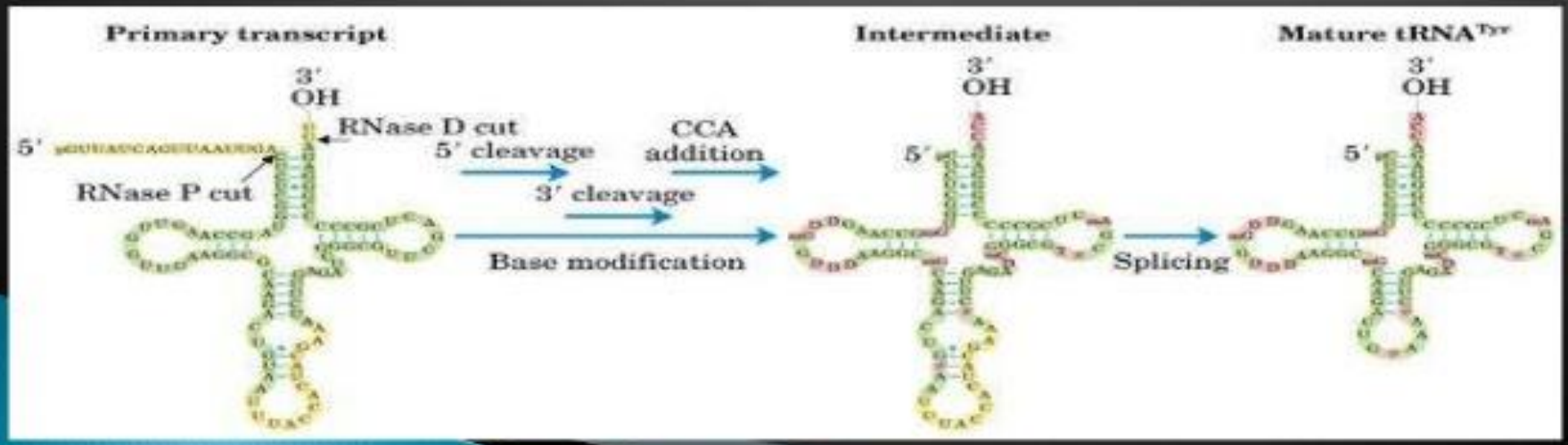


RNase P :

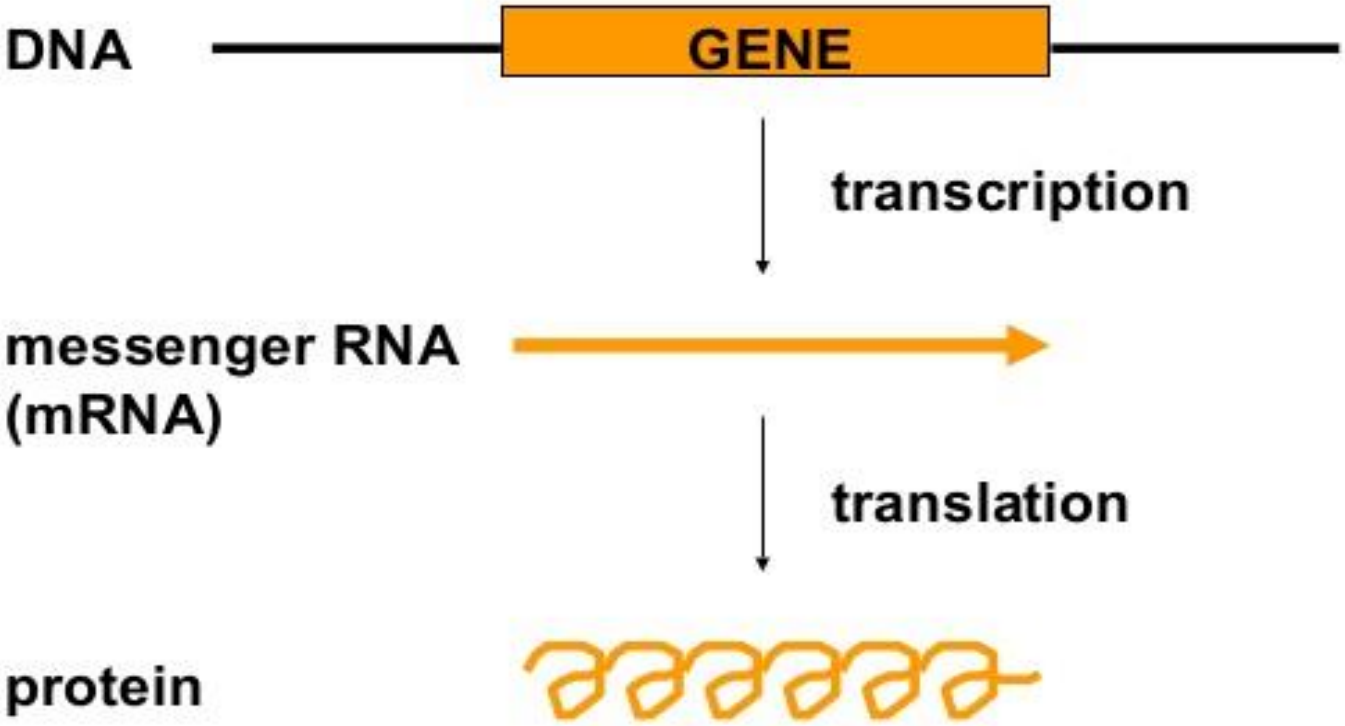
- Ribonuclease P (RNaseP), a ribonucleoprotein, is an essential tRNA processing enzyme found in all living organisms. Since its discovery almost 40 years ago, research on RNase P has led to the discovery of the catalytic properties of RNA, and of the only known, naturally occurring RNA enzymes.

Mechanism:

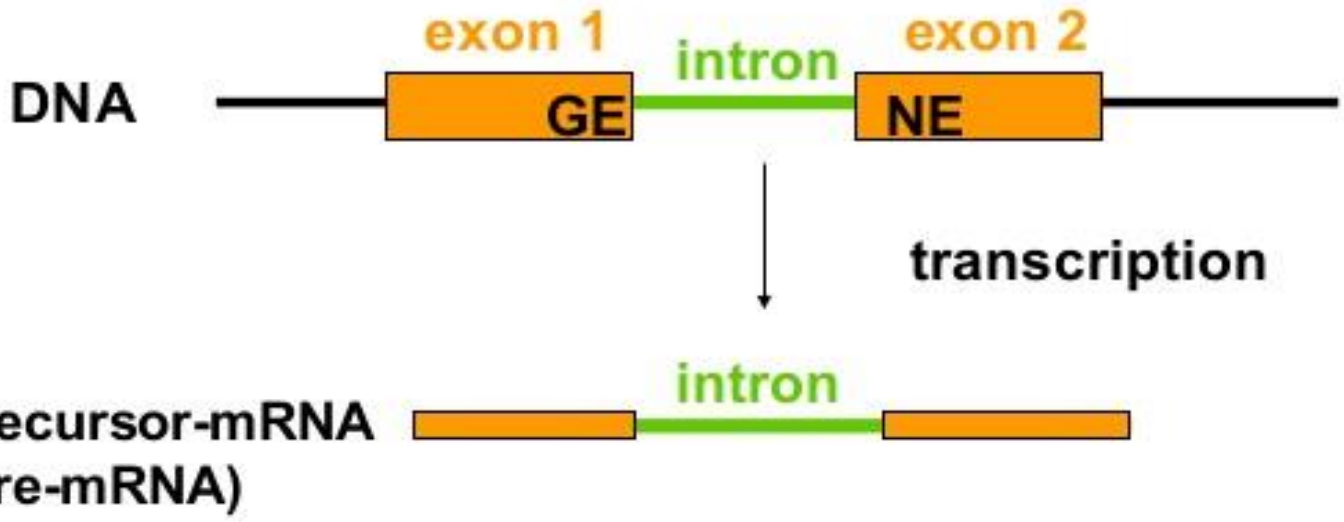
• All RNase P enzymes are ribonucleoproteins [bacteria: 1RNA + 1 protein subunit; eukaryotes: 1 RNA + many protein subunits (11 in human)],
• In Ribonulease – P, protein component is facilitates binding between RNase and t-RNA substrate.
• Requires divalent metal ions (like Mg^{2+}) for its activity.
• Endo-ribonuclease responsible for generating 5' end of matured tRNA molecules.
• Cleavage via nucleophilic attack on the phosphodiester bond leaving a 5'-phosphate and 3'-hydroxyl at the cleavage site.



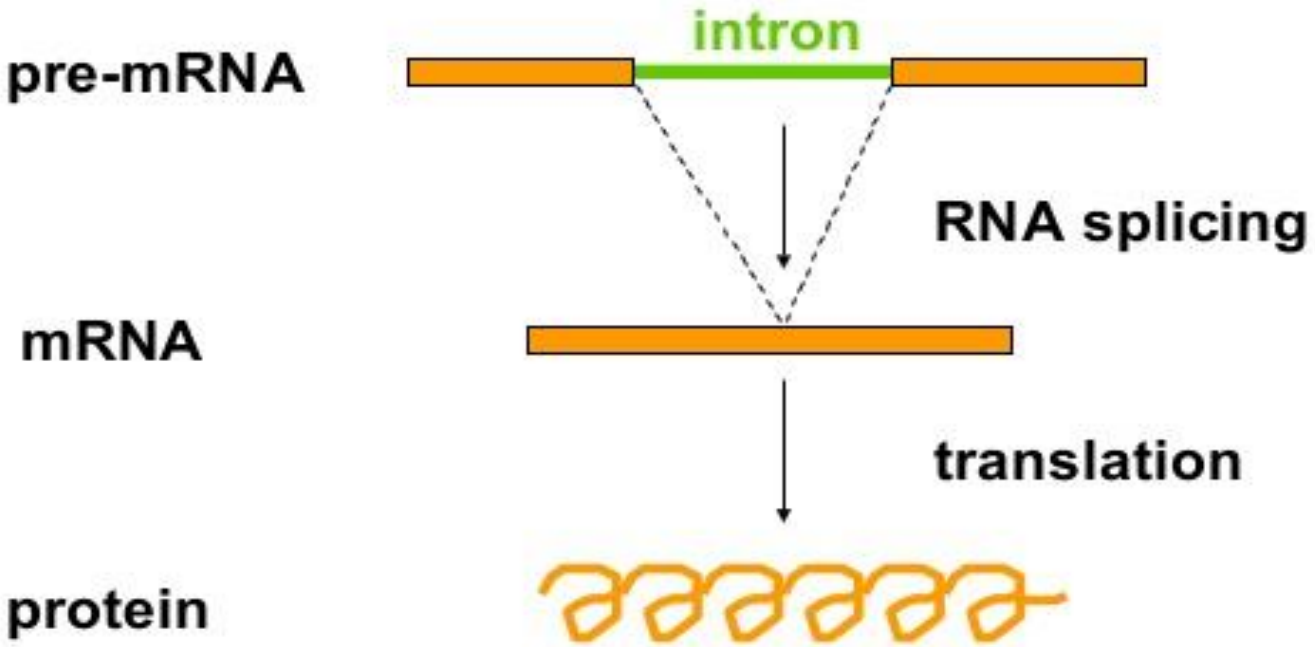
Genetic information is transferred from genes to the proteins they encode via a "messenger" RNA intermediate



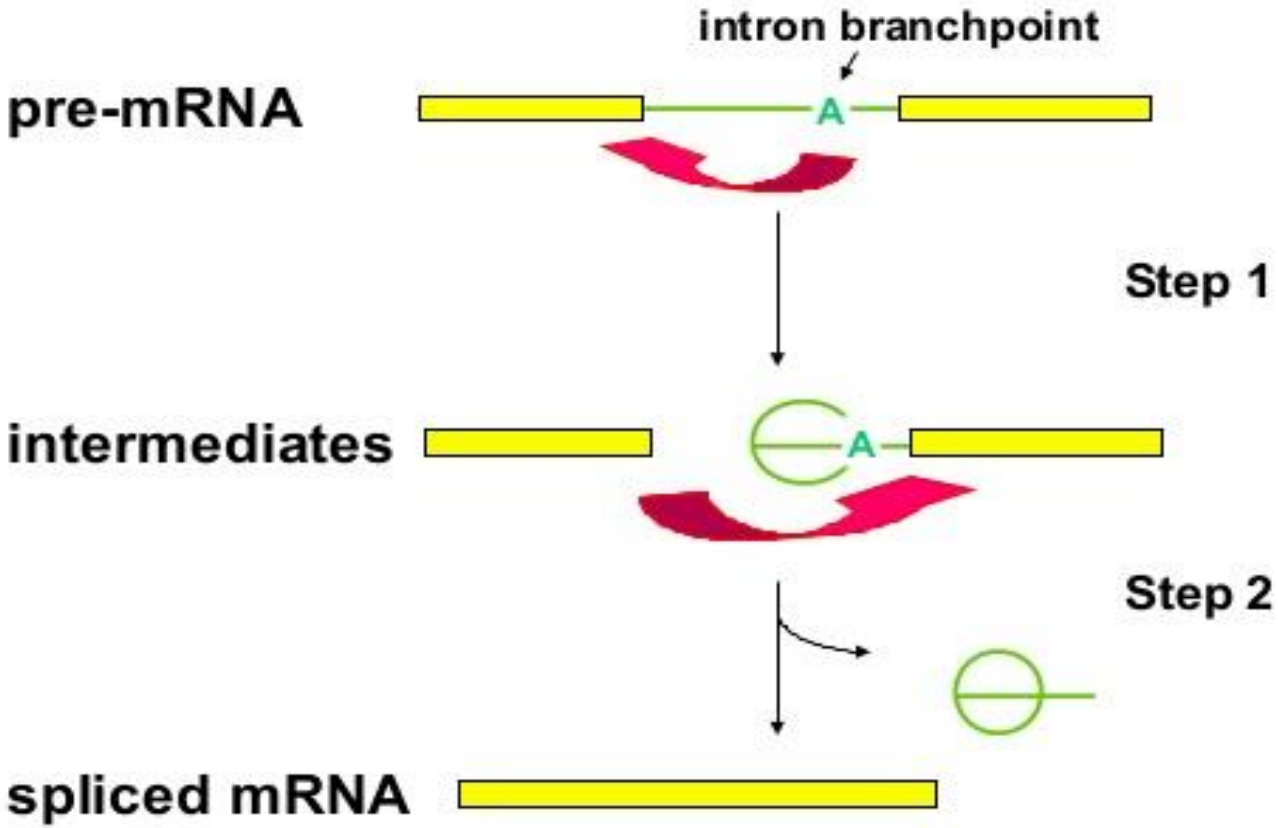
Most genes have their protein-coding information interrupted by non-coding sequences called "introns". The coding sequences are then called "exons"



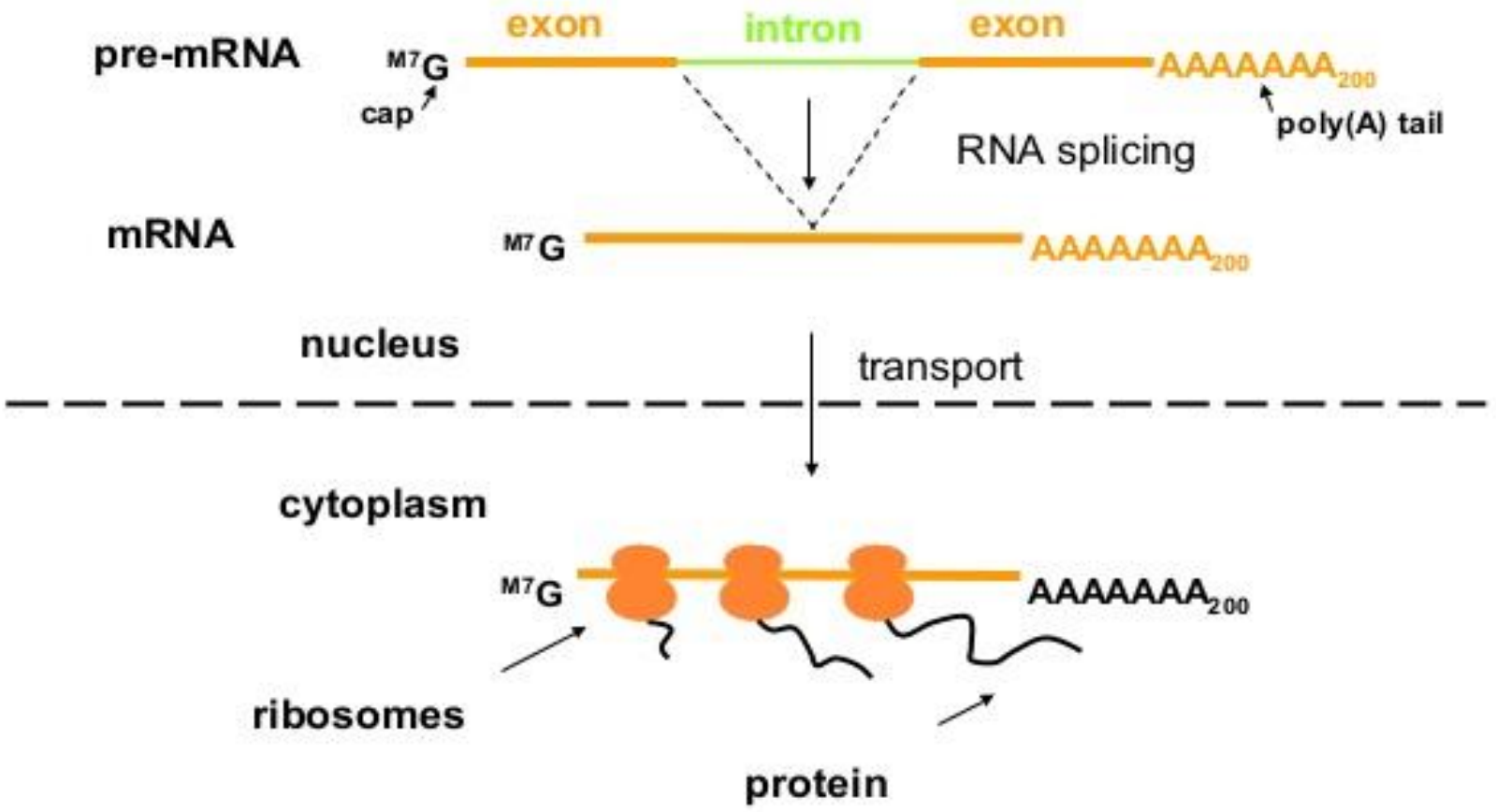
The intron is also present in the RNA copy of the gene and must be removed by a process called "RNA splicing"



Splicing a pre-mRNA involves two reactions

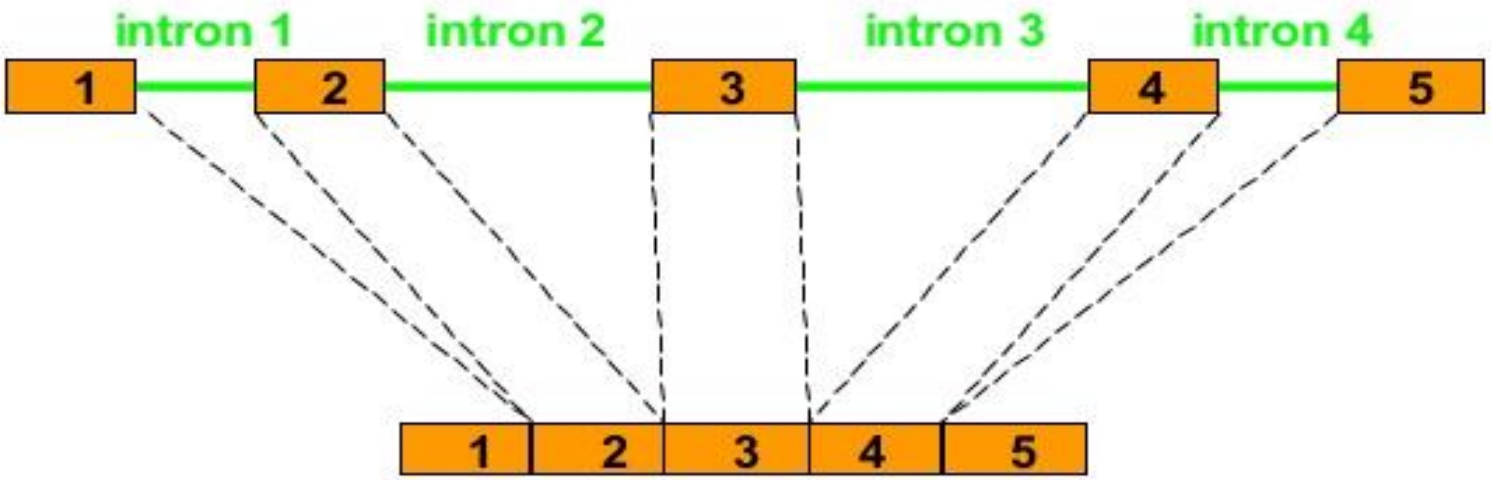


Pre-messenger RNA Processing



Alternative splicing

In humans, many genes contain multiple introns



Usually all introns must be removed before the mRNA can be translated to produce protein

Reactions naturally catalysed by RNA. Two sequential trans esterification reactions catalysed by group I. (A) ...

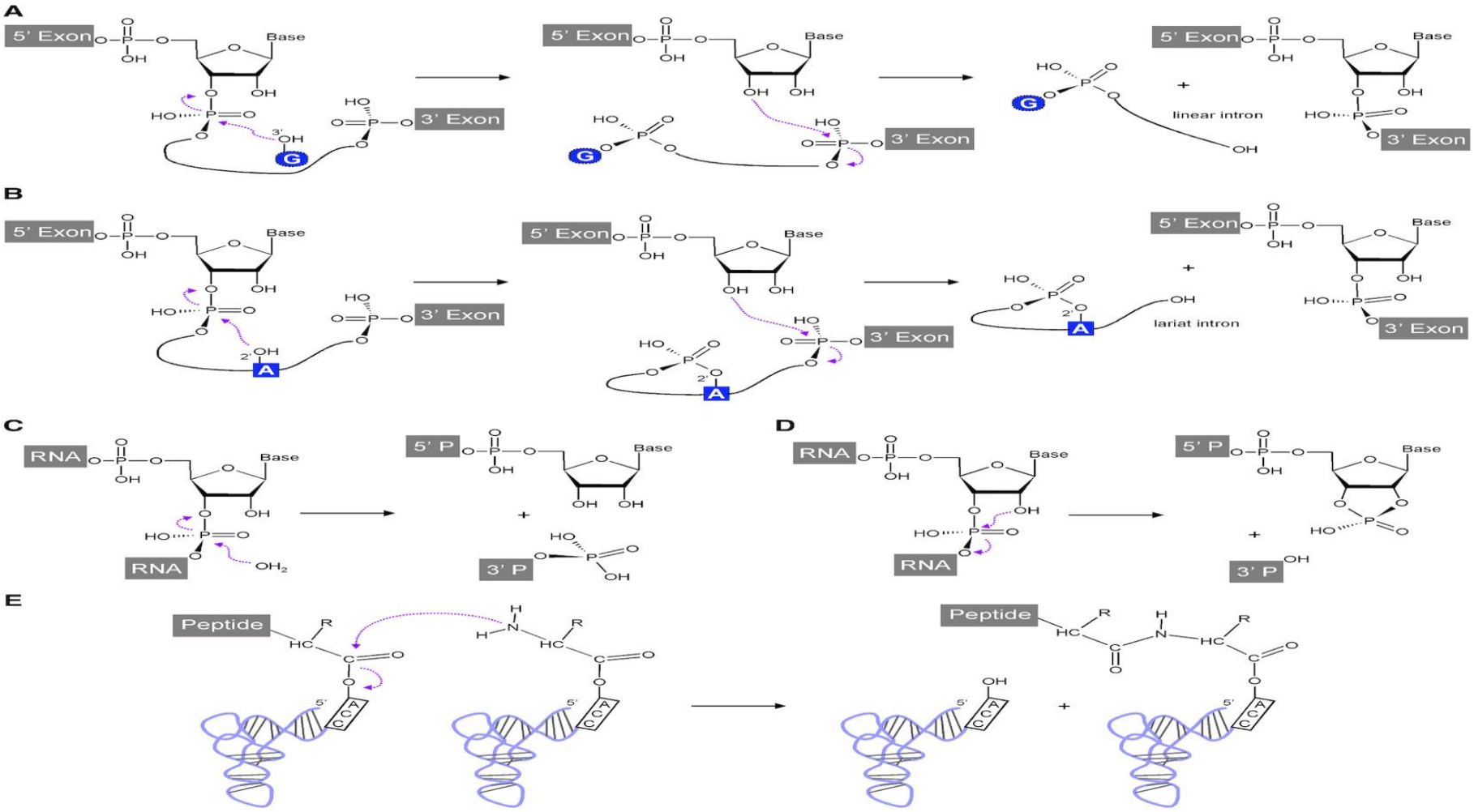
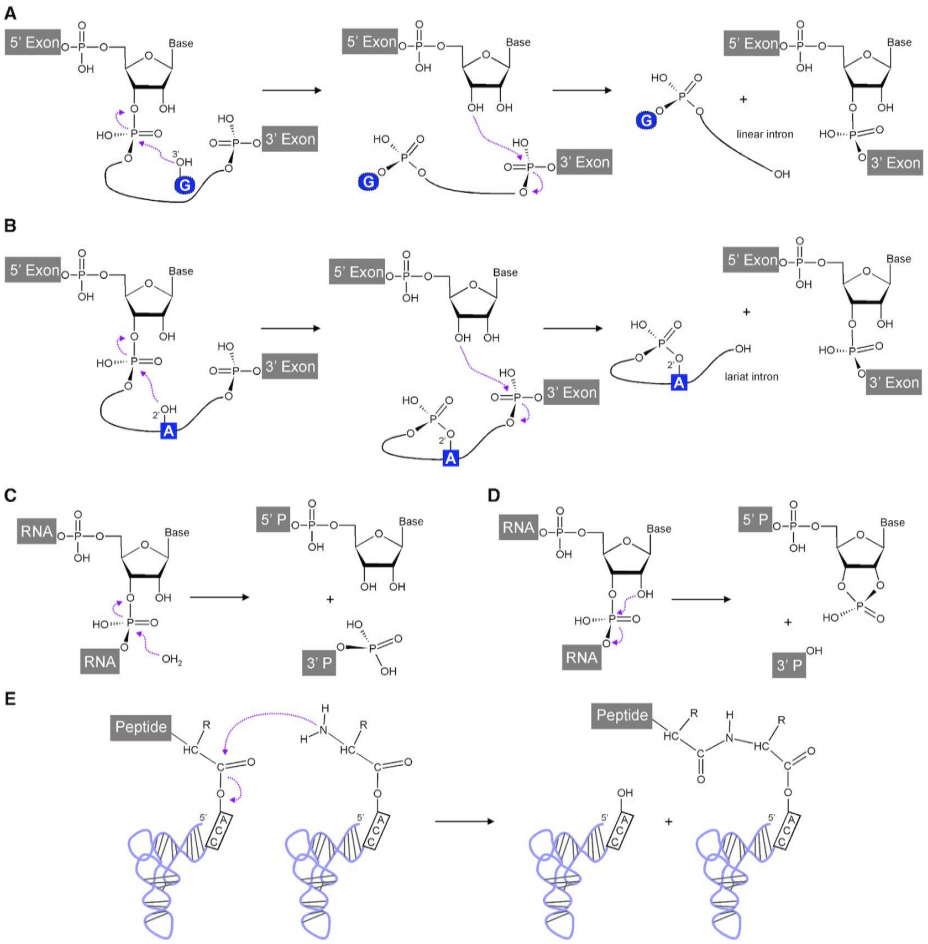


Figure 1. Reactions naturally catalysed by RNA. Two sequential transesterification reactions catalysed by group I. (A) ...



Nucleic Acids Research, Volume 47, Issue 18, 10 October 2019, Pages 9480–9494, https://doi.org/10.1093/nar/gkz737

GROUP I intron splicing

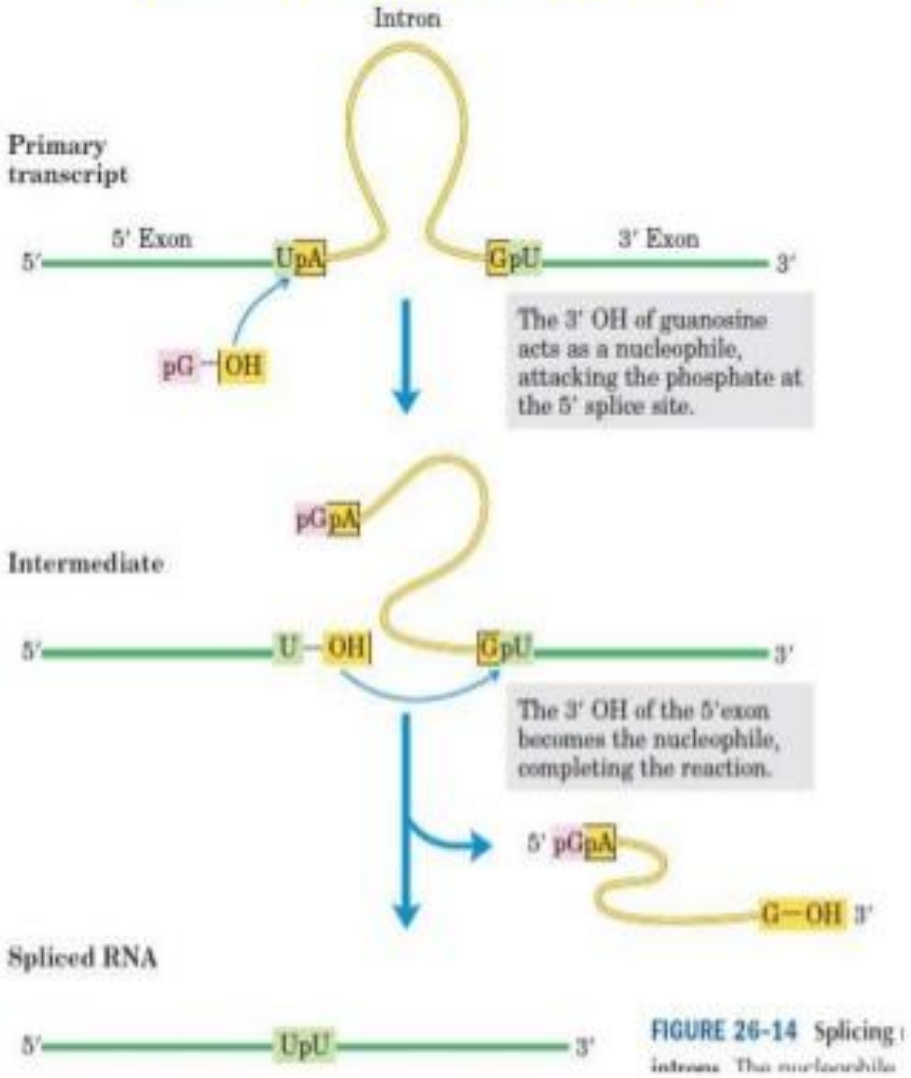


FIGURE 26-14 Splicing

GROUP II intron splicing

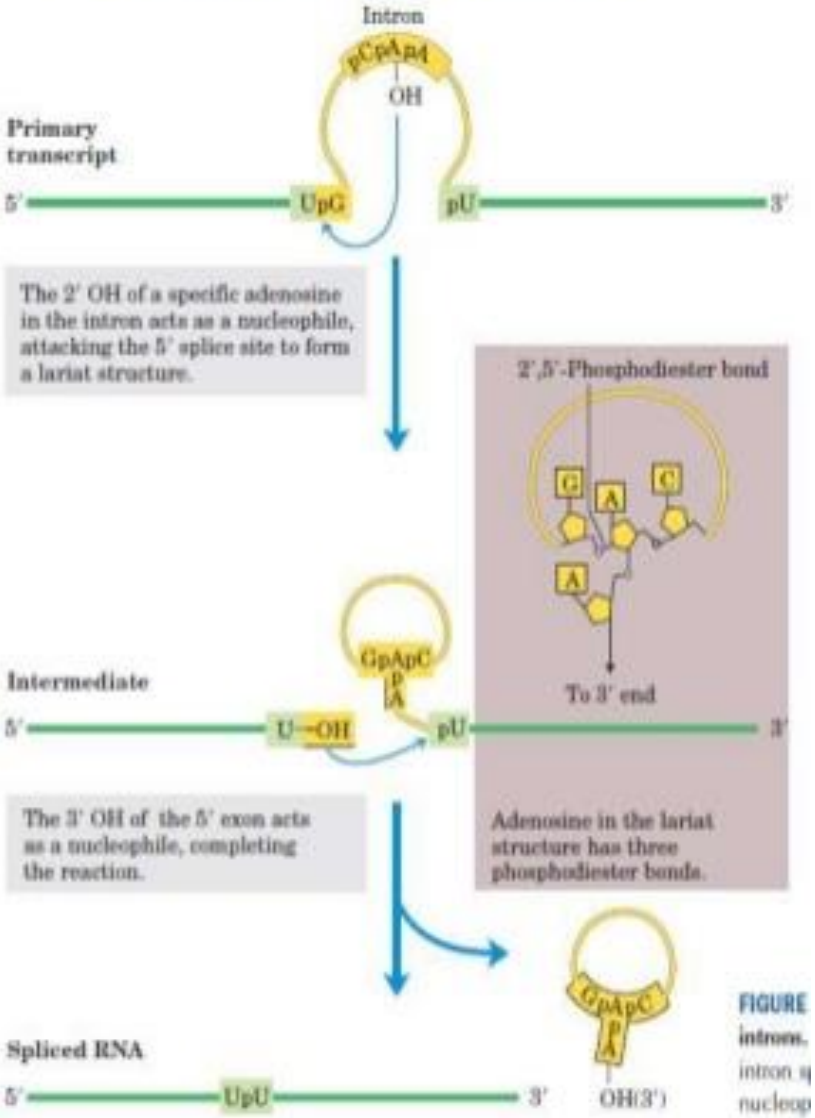
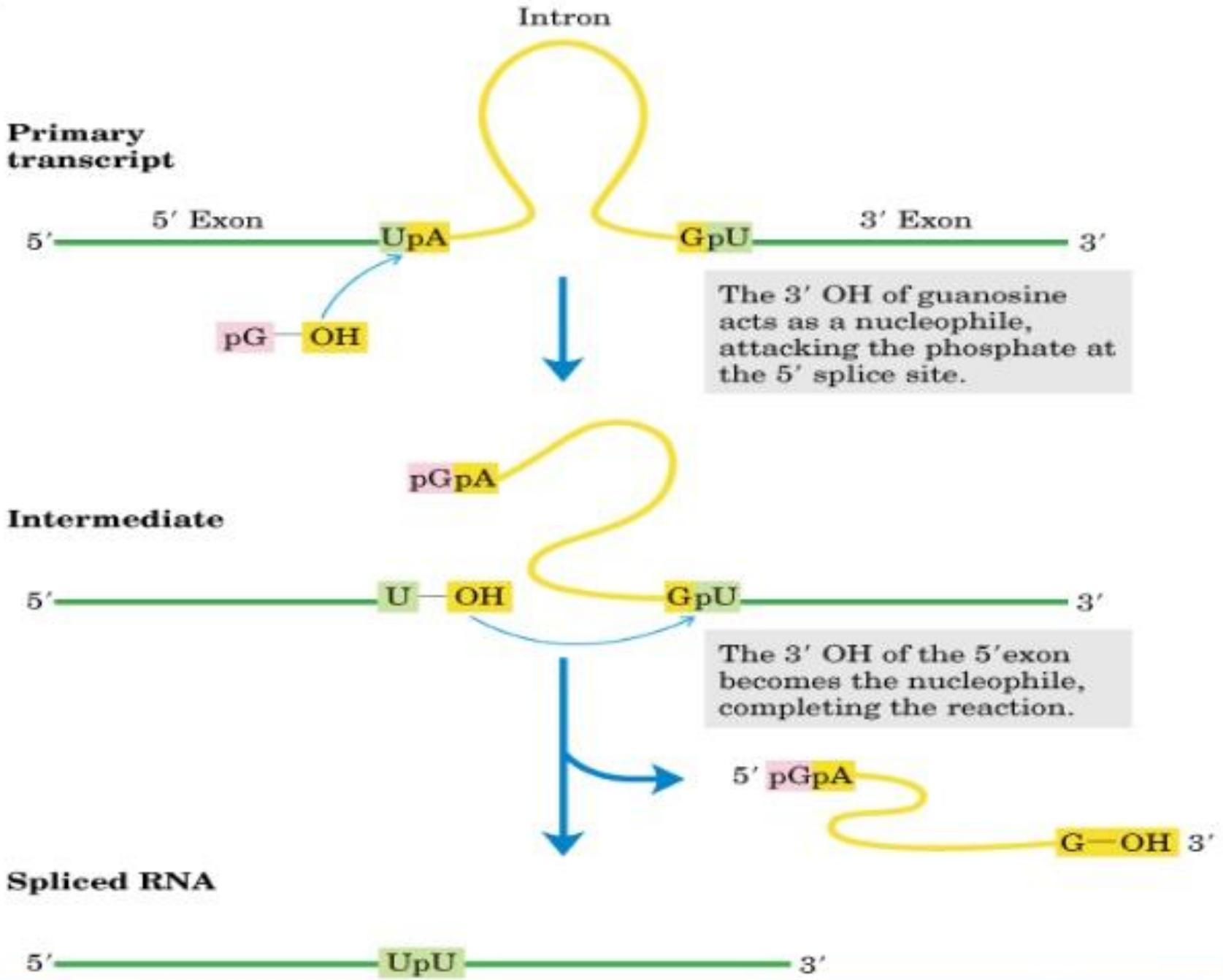
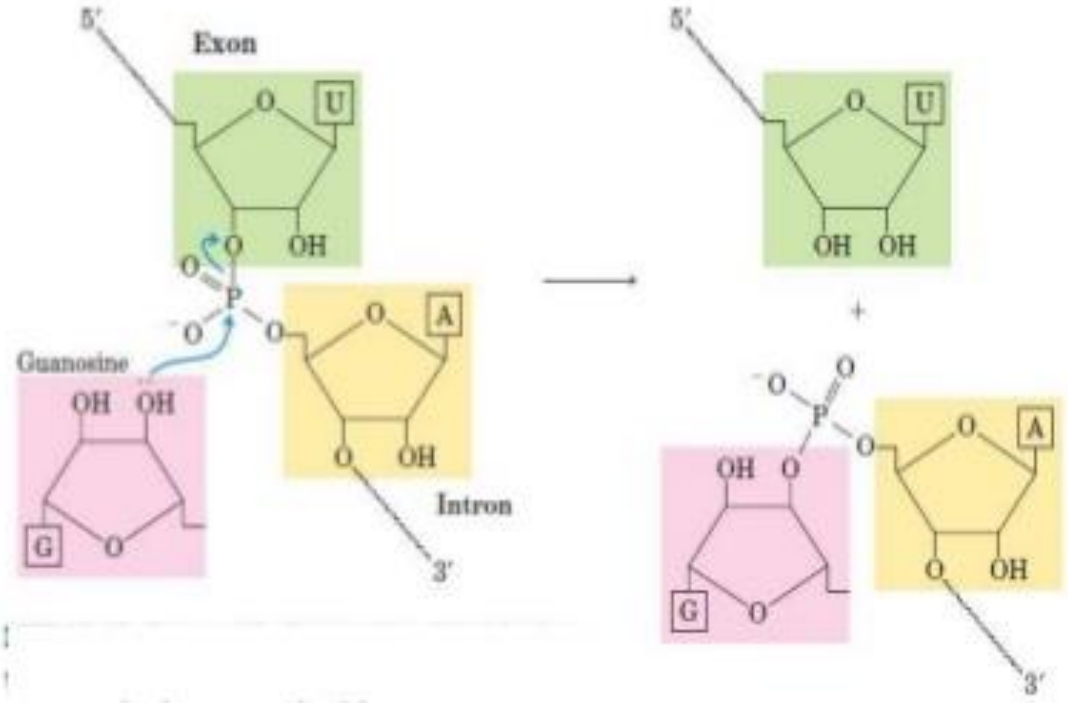


FIGURE
introns.

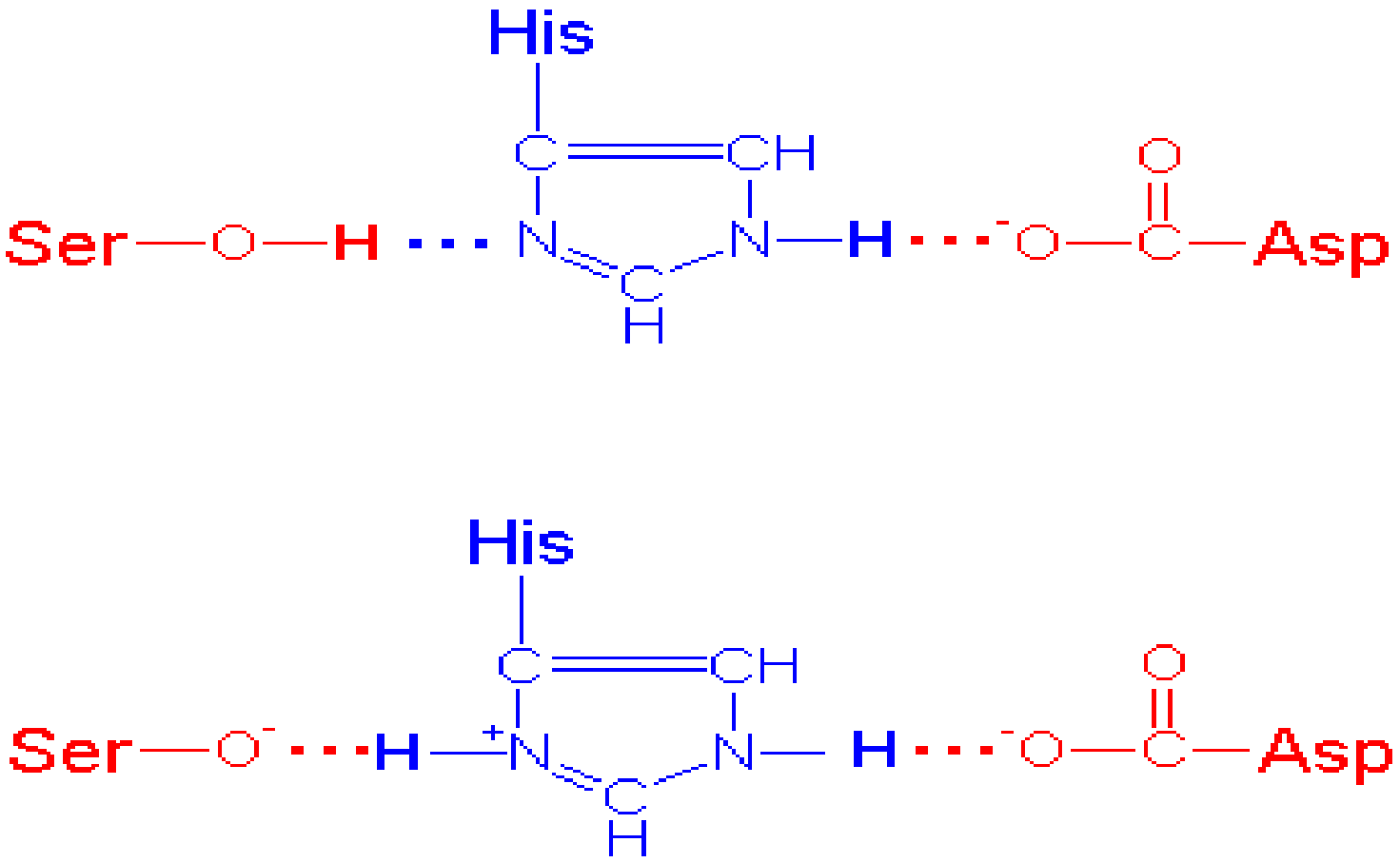

Intron
Primary transcript
5′ Exon
UpA
GpU
3′ Exon
5′
3′
pG
OH
The 3′ OH of guanosine acts as a nucleophile, attacking the phosphate at the 5′ splice site.
pGpA
Intermediate
5′
U
OH
GpU
3′
The 3′ OH of the 5′exon becomes the nucleophile, completing the reaction.
5′ pGpA
G
OH
3′
Spliced RNA
5′
UpU
3′

1. Self-splicing introns

This is a transesterification reaction in which the guanosine hydroxyl group attacks the phosphodiester bond between the 3′ end of the first exon and the first nucleotide of the intron.

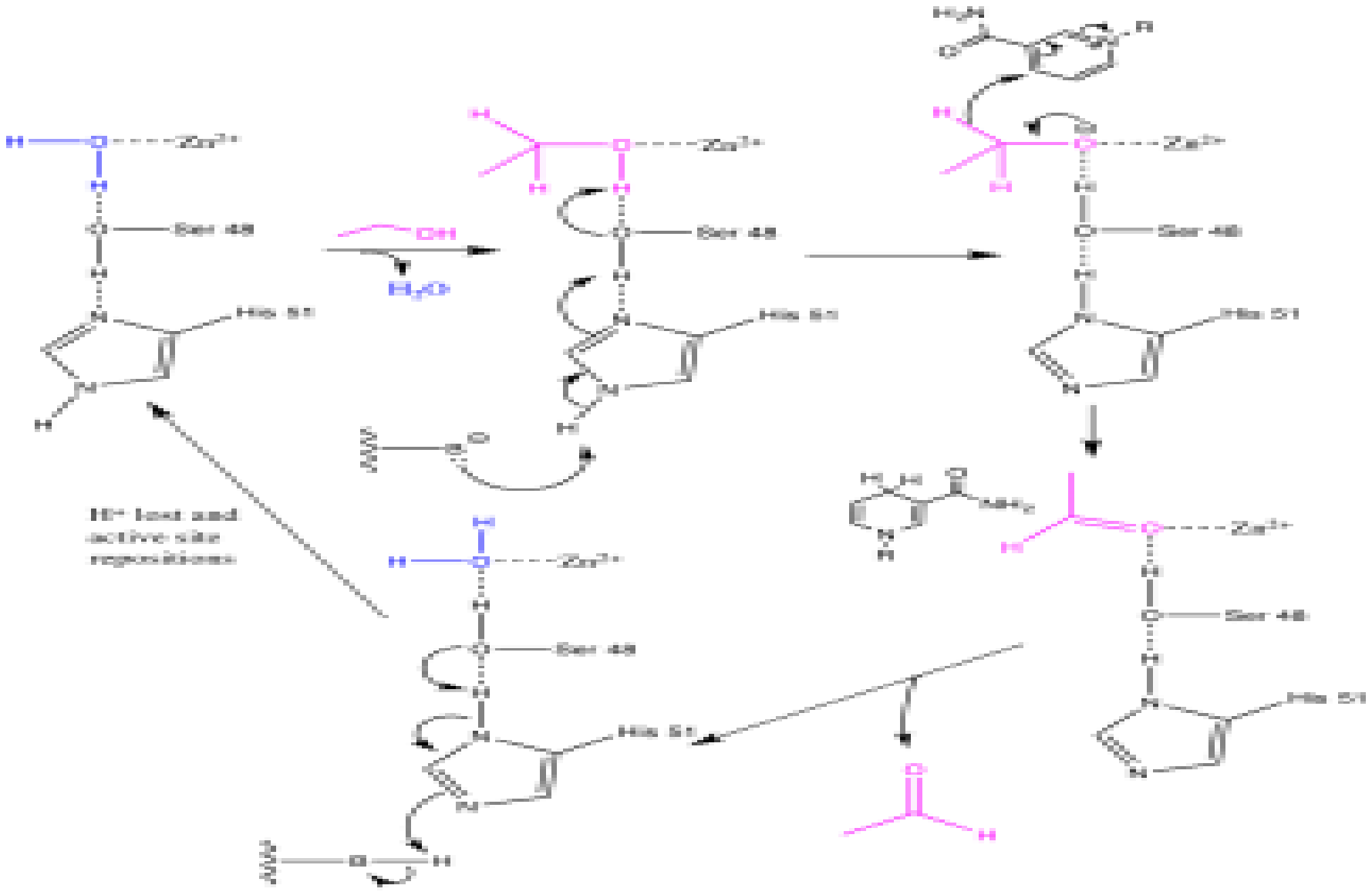


The guanosine remains attached to the 5′ end of the intron. Then, the 3′ end of the liberated exon attacks the extremity of base 413 from the intron to bring together the exon's two ends



Scheme of the charge-transfer relay network

Reactions naturally catalysed by RNA. Two sequential transesterification reactions catalysed by group I. (A) and group II (B) introns in cis. These result in joined exons and linear and lariat introns, respectively. RNA hydrolysis catalysed in trans by the M1 RNA subunit of bacterial RNase P. (C) results in a phosphate containing 5′ end of the mature tRNA as the 3′ cleavage product (3′ P) and a 3′ hydroxyl group at the 5′ cleavage product (5′ P). Small nucleolytic ribozymes undergo transesterification reactions in cis (D), in which a specific 2′-hydroxyl attacks the neighbouring 3′,5′-phosphodiester bond. This results in a 2′,3′-cyclic phosphate and a 5′ hydroxyl at the 5′ and 3′ cleavage products, respectively. (E) Peptide bond formation catalysed in the ribosomal peptidyl transferase centre. This figure was adapted from (163).


Zn2+
Ser 48
His 51
H2O
H+ lost and active site regenerated

Charge relay network

The serine in the triad is much more reactive then other serines in the protein. The serine hydrosyl is normally protonated at neutral pH, but in the enzyme Ser-195 (Ser-221) is hydrogen-bonded to His-57 (His-64), which is further hydrogen-bonded to Asp-102 (Asp-32). These three amino acids are often referred to as a **catalytic triad**.

As the serine oxygen attacks the carbonyl carbon of a peptide bond, the hydrogen-bonded His functions as a general base to abstract the serine proton, and the negatively charged Asp stabilizes the positive charge that forms on the His residue. This prevents the development of a very unstable positive charge on the serine hydroxyl and increase its nucleophilicity. The residues of the catalytic triad form a **charge-transfer relay network**.