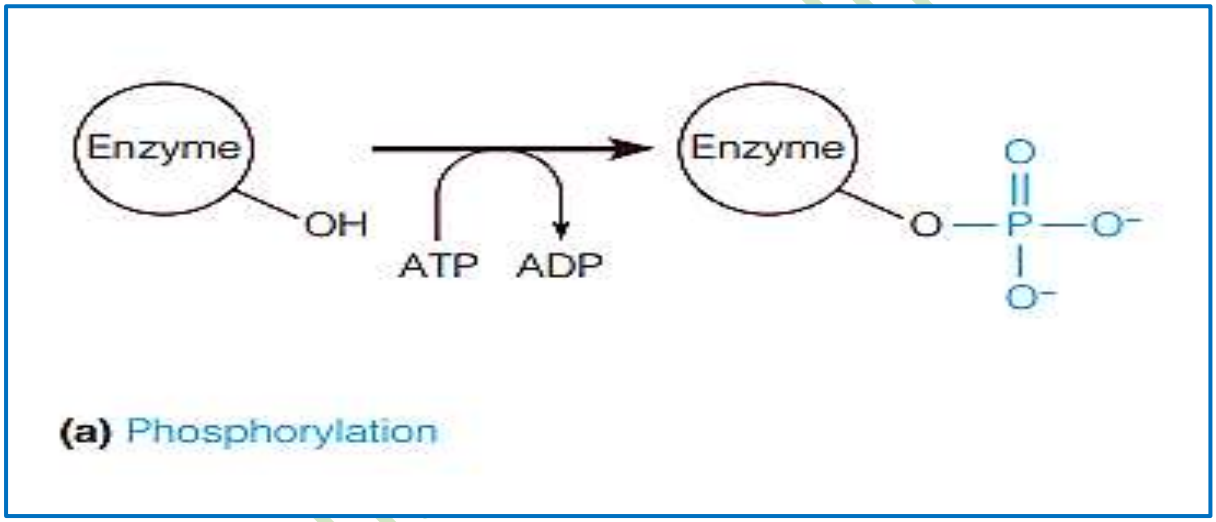
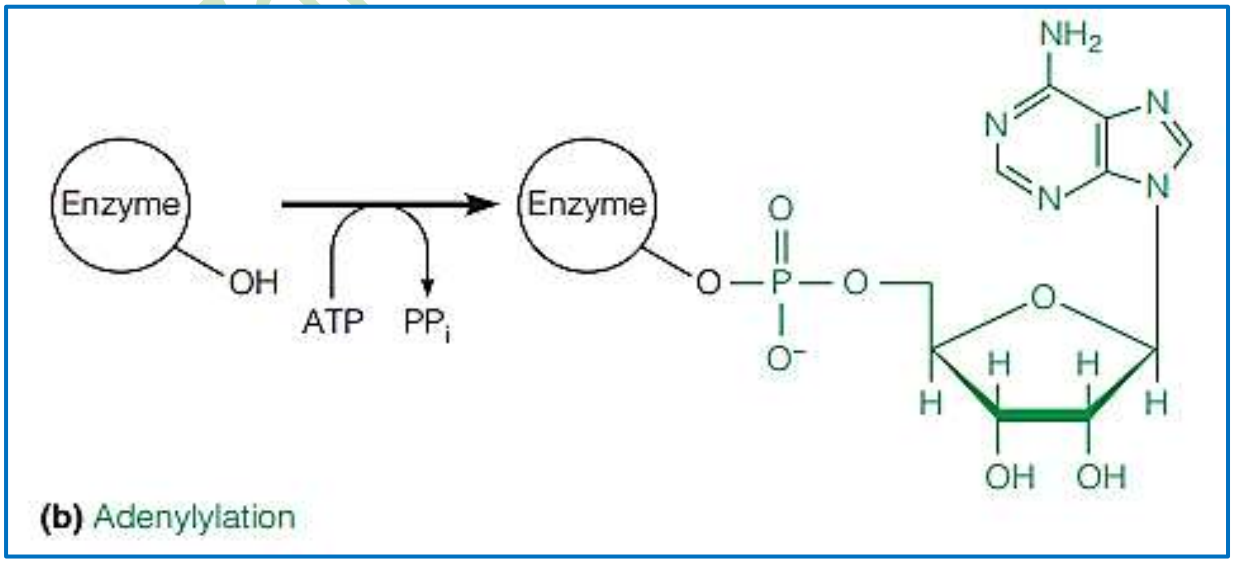
Covalent Modifications Used to *Regulate Enzyme Activity*

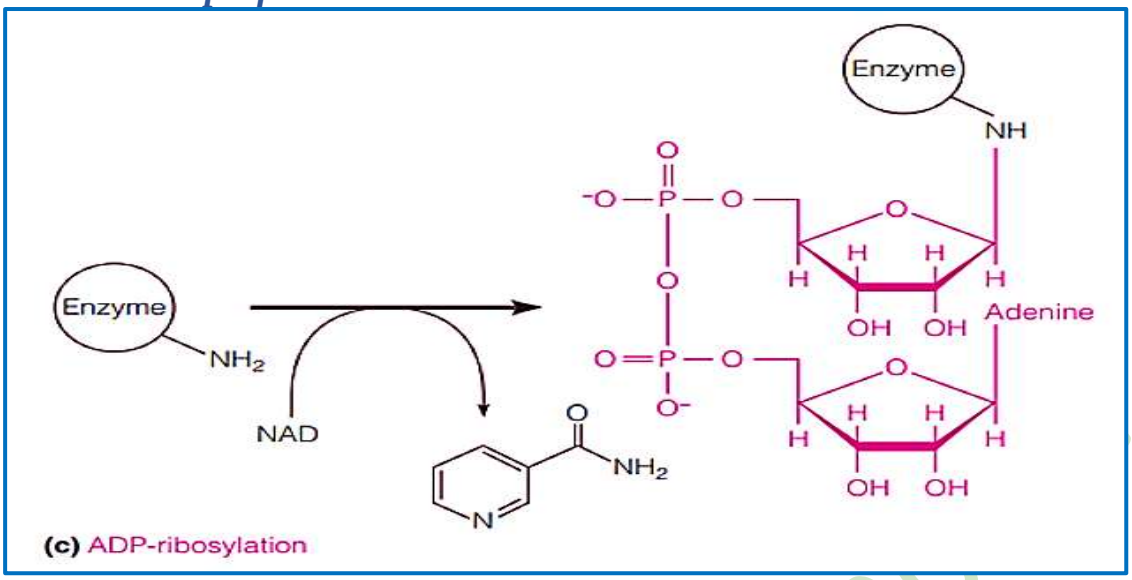
A number of kinds of covalent modifications are commonly used to regulate enzyme activity (Figure 11.51).

The most widespread is phosphorylation or dephosphorylation of various amino acid side chains (serine, threonine, tyrosine, and histidine, for example).

Other covalent modifications include adenylation, the transfer of an adenylate moiety from ATP; ADP-ribosylation, the transfer of an ADP-ribosyl moiety from and acetylation, the transfer of an acetyl group from acetyl-coenzyme A (see Table 11.5).







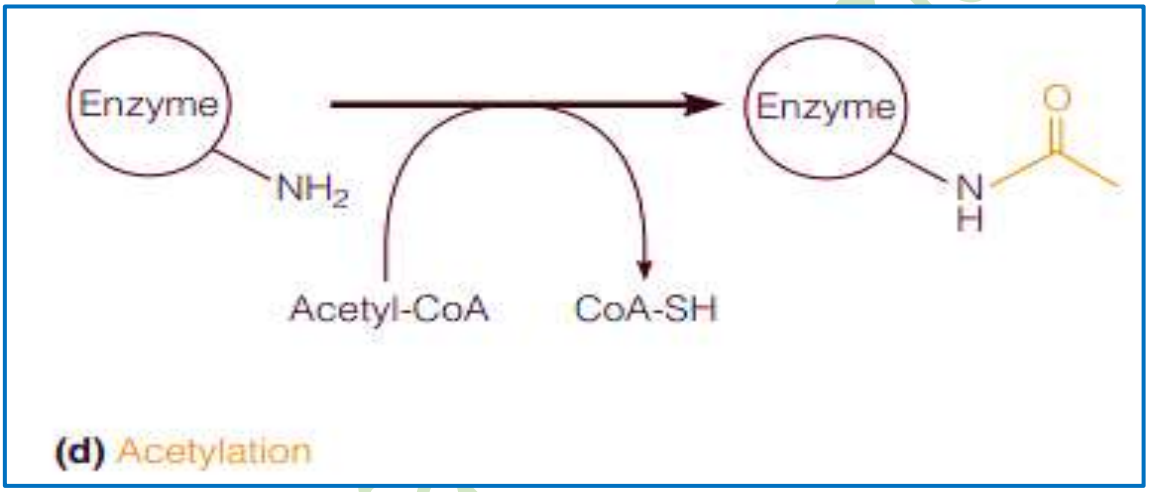


FIGURE 11.51 ***Four types of covalent modifications that control the activities of enzymes.*** The target residue for phosphorylation oadenylation is usually serine, threonine, or tyrosine, whereas ADP-ribosylation can involve arginine, glutamate, aspartate, or a modified histidine residue. N-acetylation involves a reaction between a lysine side chain and acetyl-coenzyme A.

The majority of enzymes, and their associated metabolic and signaling pathways, are regulated by reversible phosphorylation.

Protein kinases are ATP-dependent enzymes that add a phosphoryl group to the group of Tyr, Ser, or Thr on some target proteins (Figure 11.52).

This process is made reversible by the second class of enzymes, called phosphatases, which hydrolyze the resulting side chain phosphate esters, releasing.

Protein phosphorylation and acetylation are part of complex regulatory pathways, frequently under hormonal control. The majority of enzymes, and their associated metabolic and signaling pathways, are regulated by reversible phosphorylation. Protein kinases are ATP-dependent enzymes that add a phosphoryl group to the group of Tyr, Ser, or Thr on some target proteins (Figure 11.52).

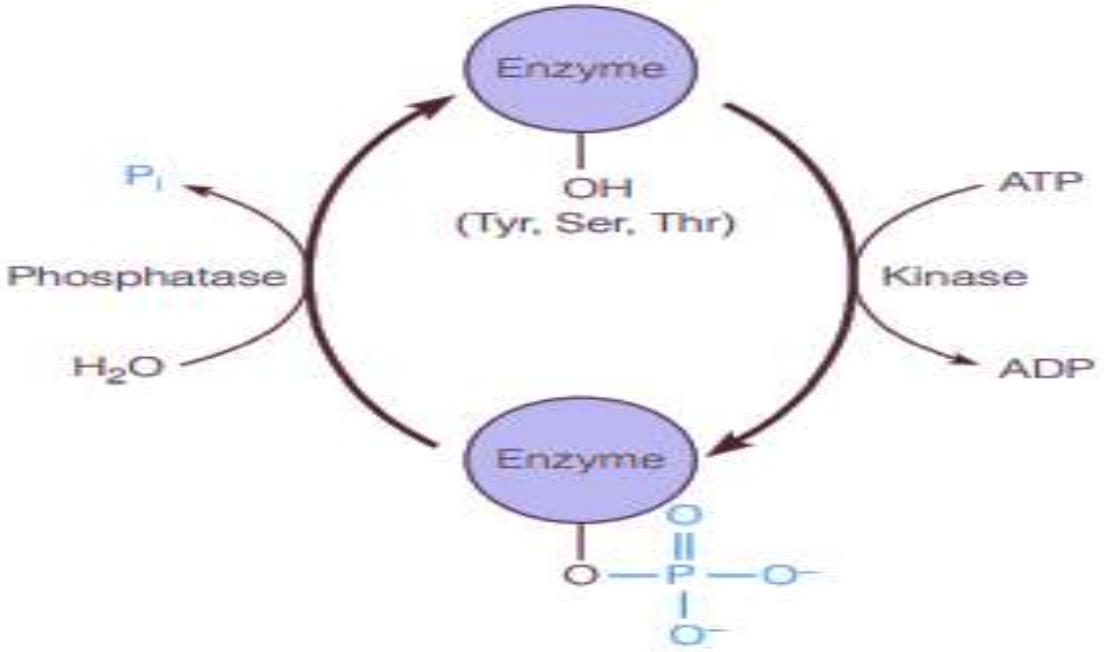


FIGURE 11.52 ***Reversible covalent modification by kinases/ phosphatases.*** The target residues for ATP-dependent phosphorylation by kinases are serine, threonine, or tyrosine. The phosphoprotein is dephosphorylated by a phosphatase-catalyzed hydrolysis reaction.

Pancreatic Proteases: *Activation by Cleavage*

Some enzymes, such as pancreatic proteases, are irreversibly switched on by proteolytic cleavage

An important example of covalent enzyme activation, proteolytic cleavage. These include a number of enzymes—for example, trypsin, chymotrypsin, elastase, and carboxypeptidase

They are secreted through the pancreatic duct into the duodenum of the small intestine in response to a hormone signal generated when food passes from the stomach.

They are not synthesized in active form because potent proteases free in the pancreas would digest the pancreatic tissue.

They are made as slightly longer, called **zymogens.** Included: chymotrypsinogen, proelastase, and procarboxypeptidase, respectively. The zymogens must be cleaved proteolytically in the intestine to yield the active enzymes.

The cleavage of zymogens to active enzymes is diagrammed in Figure 11.53.

The first step is the activation of trypsin removed from the N-terminal end of trypsinogen by enteropeptidase, a protease secreted by duodenal cells. This action yields the active trypsin, which then activates the other zymogens by specific proteolytic cleavages.

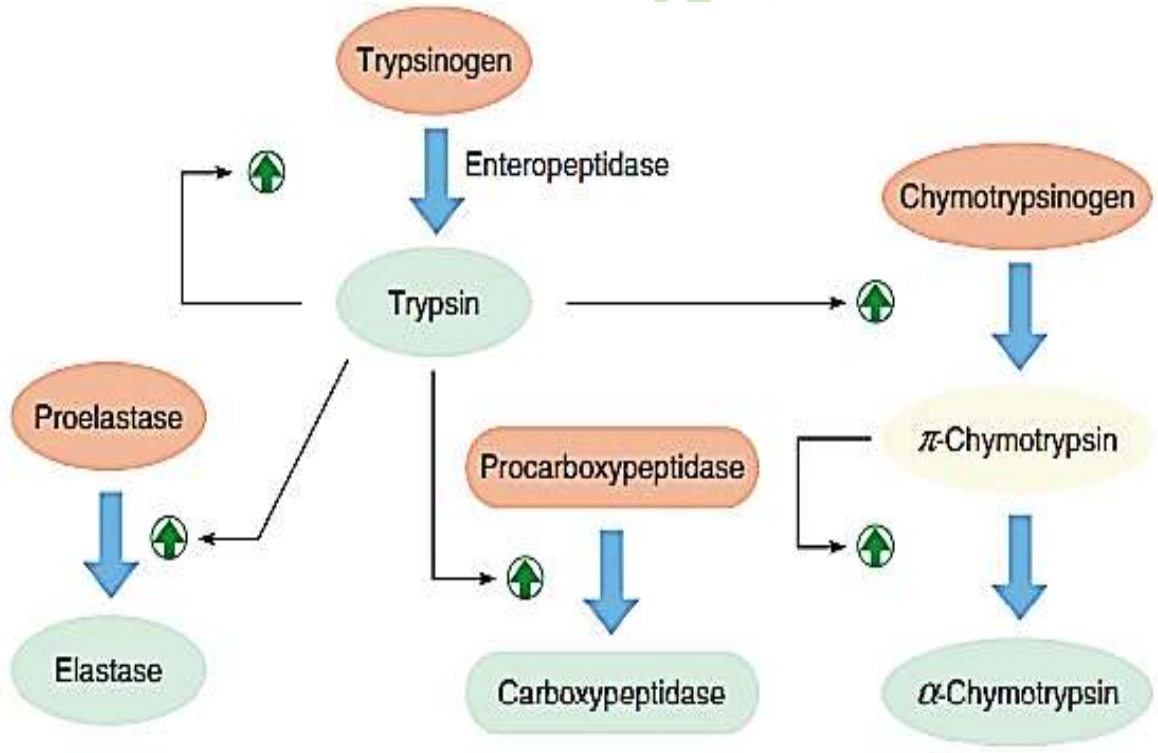


FIGURE 11.53 ***Zymogen activation by proteolytic cleavage.*** This schematic view shows the activation of pancreatic zymogens, molecules that become catalytically active when cleaved. Zymogens are shown in orange and active proteases are in yellow or green. The difference between -chymotrypsin and -chymotrypsin is shown in Figure 11.54.

Non-protein Biocatalysts:

Catalytic antibodies (Abzyme)

Antibodies show remarkably high specificity in binding to their antigens.

Enzymes bind most strongly to the transition state in a reaction.

What happens if we make antibodies against molecules that are structurally analogous to the transition state of a particular substrate?

The answer is that:

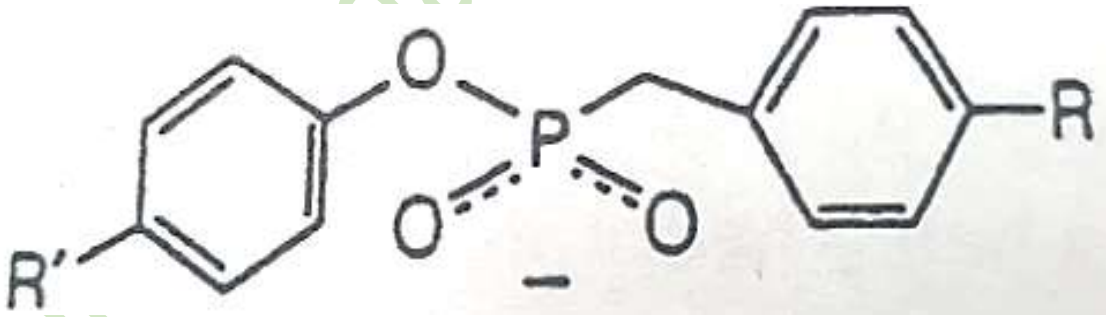
These antibodies act like enzymes, so they are now often called **abzymes.**

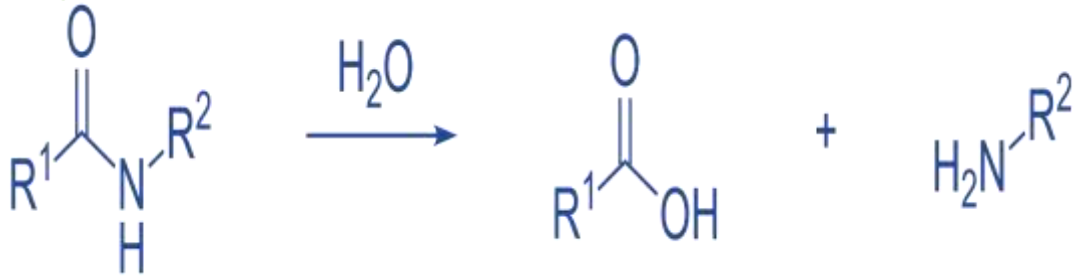
Suppose, for example, we wish to make an antibody that will function as a catalyst for the hydrolysis of esters.

Example:

Hydrolysis of amides, a tetrahedral transition state is required in ester hydrolysis.

A kind of molecule that mimics such a structure is the following:





By using various kinds of molecules as antigens, it has been possible to produce abzymes catalyzing a number of the classes of reactions in Table 11.7. in some cases, reaction rates as much as 10^7 times the uncatalyzed rate have been obtained.

Abzyme (from antibody and enzyme), also called catmab (from catalytic monoclonal antibody), and most often called catalytic antibody or sometimes catab,[is a monoclonal antibody with catalytic activity.

For many years, the major difficulty in generating catalytic antibodies directed toward specific compounds or functional groups was the necessity of using the immune system of some animals to make the selection.

More recently, however, selection systems have been developed that circumvent this requirement.

The basic idea is that randomly rearranged Fab fragments (see Chapter 7) are cloned and the mixture is subjected to selection by chemical affinity to the desired molecule or structure.

Such techniques have allowed the development of abzymes. directed toward synthetic molecule substrates that would be very difficult to present as antigens to an in vivo system because of toxic effects.

Catalytic antibodies are becoming of considerable importance in synthetic organic chemistry.

A major problem in the in vitro synthesis of complex organic molecules is that of obtaining the correct stereochemistry. The remarkable stereospecificity exhibited by enzymes (including abzymes) has enormously aided in some such syntheses. It seems at this point that biochemists have only begun to explore the possibilities of engineering enzymes for specific purposes.

Catalytic Nucleic Acids (Ribozyme , DNAzymes)

Ribozymes, a class of ribonucleic acids, function as biological catalysts.

Ribozymes are catalytically active RNA molecules or RNA–protein complexes,

in which solely the RNA provides the catalytic activity.

The term ribozyme refers to the enzymatic activity and ribonucleic acid nature at the same time.

Ribozymes are found in the genomes of species from all kingdoms of life.

Indeed, it was assumed that all biochemical catalysis was carried out by proteins for many years. But biochemistry is full of surprises, a research performed in the 1980s revealed something wholly unexpected: ***Some RNA molecules***, called ***ribozymes***, can ***act as enzymes.***

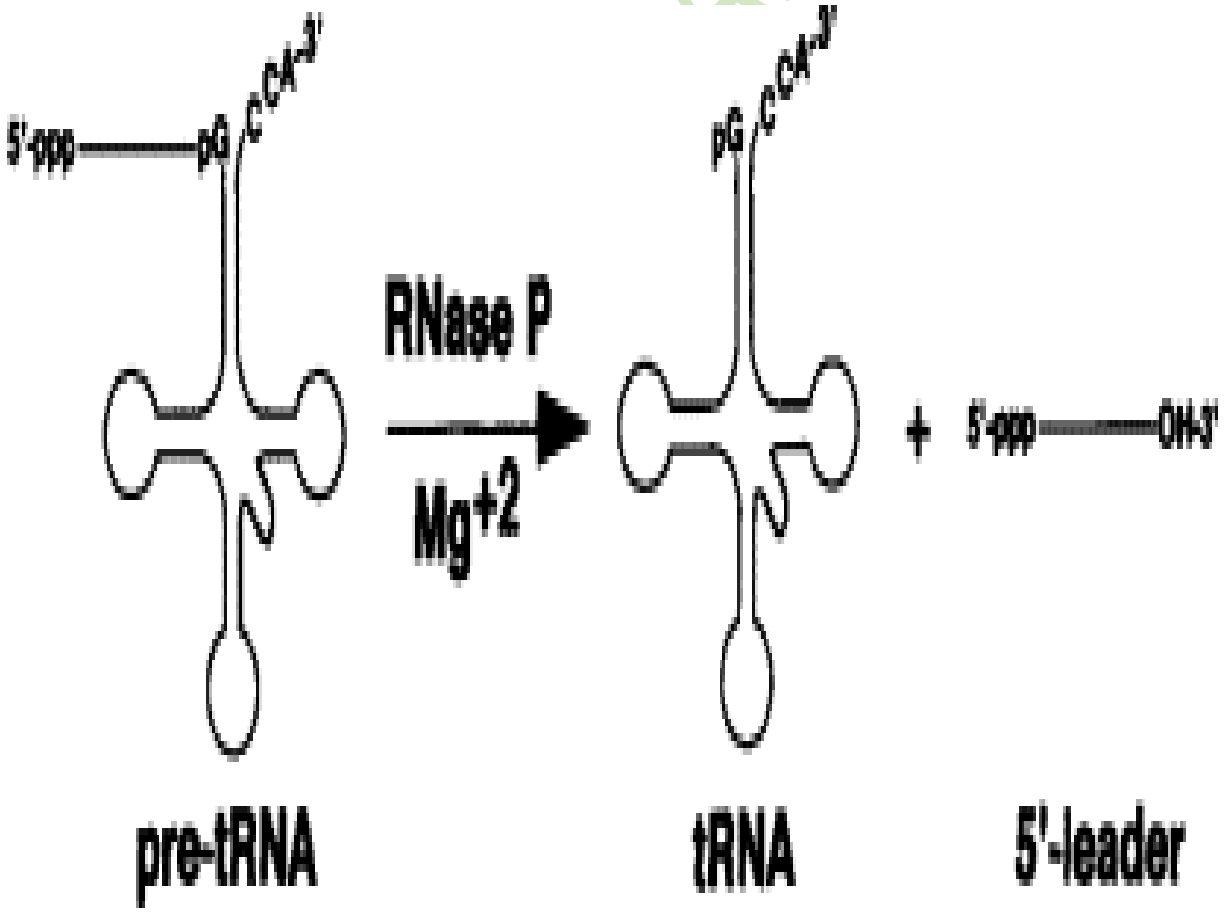
It had been known for some time that active **ribonuclease P** contained - **a protein and RNA** but it was widely assumed that the active site resided on the protein portion.

The first hint that RNA might have catalytic activity came from studies of ribonuclease P, an enzyme that cleaves the precursors of tRNAs to yield the functional tRNAs (Figure 11.40 and Chapter 27).

Ribonuclease P (**EC 3.1.26.5, RNase P**) is an RNA-cleaving ribonuclease. RNase P differs from other RNases in that it is a ribozyme, which is a ribonucleic acid that functions as a catalyst in the same way that as an enzyme that is made of a protein. Its (RNase P) function is to act on the maturation of tRNA by clipping an extra or primary RNA sequence in its molecules.

However, careful studies of the isolated components by Sidney Altman and coworkers in 1983 revealed a fact: Whereas the protein component alone was wholly inactive, the RNA by itself, if provided with either a sufficiently high concentration of magnesium ion or a small amount of magnesium ion plus the small basic molecule ***spermine****, was capable of catalyzing the specific cleavage of ***pre-tRNAs.***

Furthermore, the RNA acted like a true enzyme, being unchanged in the process and obeying Michaelis–Menten kinetics. The addition of the protein portion of ribonuclease P does enhance the activity (K_{cat} is markedly increased) but is in no way essential for either substrate binding or cleavage.



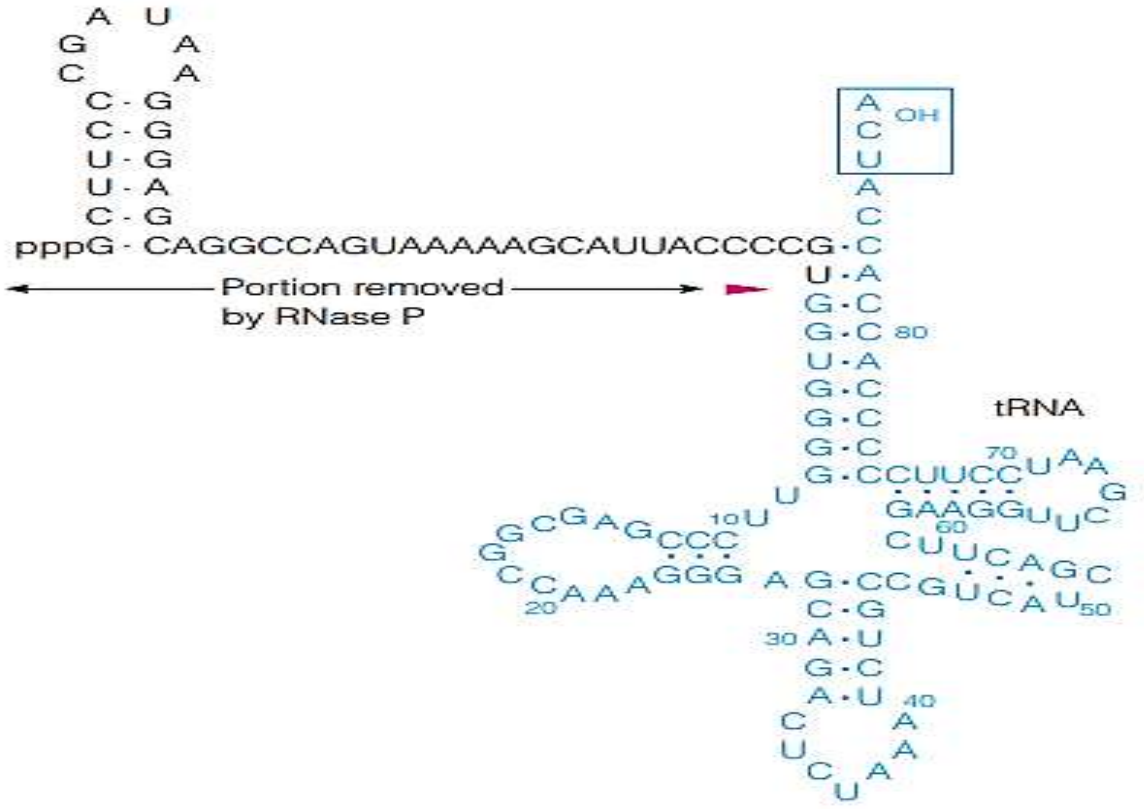


FIGURE 11.40 Cleavage of pre-tRNA by ribonuclease P. The production of tRNA from pre-tRNA is catalyzed by an **RNA–protein complex** called ribonuclease P (RNase P). The portion removed from tRNA is shown in **black**, and the resulting tRNA is in blue. The RNA portion of ribonuclease P can, by itself catalyze the hydrolysis of the specific **phosphodiester bond** indicated by the magenta wedge. The 3' terminal-OH group is shown as a subscript to the 3' terminal-OH adenosine.

DNAzymes

Like RNA, single-stranded DNA can form complex tertiary structures required for specific binding to a ligand/ (or) substrate and catalytic activity. Given the chemical similarities between DNA and RNA, it is reasonable to ask whether DNA can carry out meaningful biological catalysis. So far, no naturally occurring catalytic DNA or ***DNAzyme*** has been discovered in cells.

Like ribozymes, ***DNAzymes*** have been shown to catalyze a diverse set of reactions, with significant rate enhancements (Table 11.11). For example, the types of reactions catalyzed by DNAzymes include hydrolysis (RNA and DNA cleavage), bond cleavage, and photolytic repair of damaged DNA. Because DNA has greater chemical stability than do RNA and peptides, there is much current interest in developing DNAzymes as therapeutics, diagnostics, and biosensors.

TABLE 11.11 Examples of reaction types and rate enhancements

Reaction Type	k_{cat} (min^{-1})	Rate Enhancement
Various RNA transesterifications	0.007–4.3	10^5–10^8
DNA cleavage	0.05–0.2	10^7–10^8
Porphyrin metallation	1.3	10^3
DNA ligation	0.0001–0.07	10^2–10^5
Adenylylation	0.005	10^{10}
N-Glycosyl cleavage	0.2	10^6
Phosphorylation	0.012	10^9

From Cellular and Molecular Life Sciences 59:596–607, G. M. Emilsson and R. R. Breaker, Deoxyribozymes: New activities and new applications, © 2002, with kind permission from Springer Science+Business Media B.V