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ENZYMES IN MODIFICATION POLYNUCLEOTIDE, PHOSPHORYLASE, DNASE AND THEIR MECHANISM OF ACTION

Polynucleotide phosphorylase:

- Polynucleotide phosphorylase was first discovered from extracts of *Azotobacter agile* by Grunberg-Manago and Ochoa.
- Polynucleotide phosphorylase (PNPase) catalyzes the synthesis of long chain polyribonucleotides (RNA) in 5' to 3' direction from nucleotide diphosphates as precursors and reversibly catalyzes phosphorolytic cleavage of polyribonucleotides in 3' to 5' direction with a release of orthophosphate in presence of inorganic phosphate.
- PNPase is a bifunctional enzyme and functions in mRNA processing and degradation inside the cell.
- Structural and physiochemical studies in enzymes showed that it is formed of subunits. The arrangements of the subunits may vary from species to species which would alter their properties.
- This enzyme can catalyze not only the synthesis of RNA from the mixtures of naturally occurring ribonucleoside diphosphates, but also that of non-naturally occurring polyribonucleotides.

Mechanism of action:

As mentioned earlier, polynucleotide phosphorylase is a bifunctional enzyme. The mechanism of action of this enzyme can be represented by following reactions:

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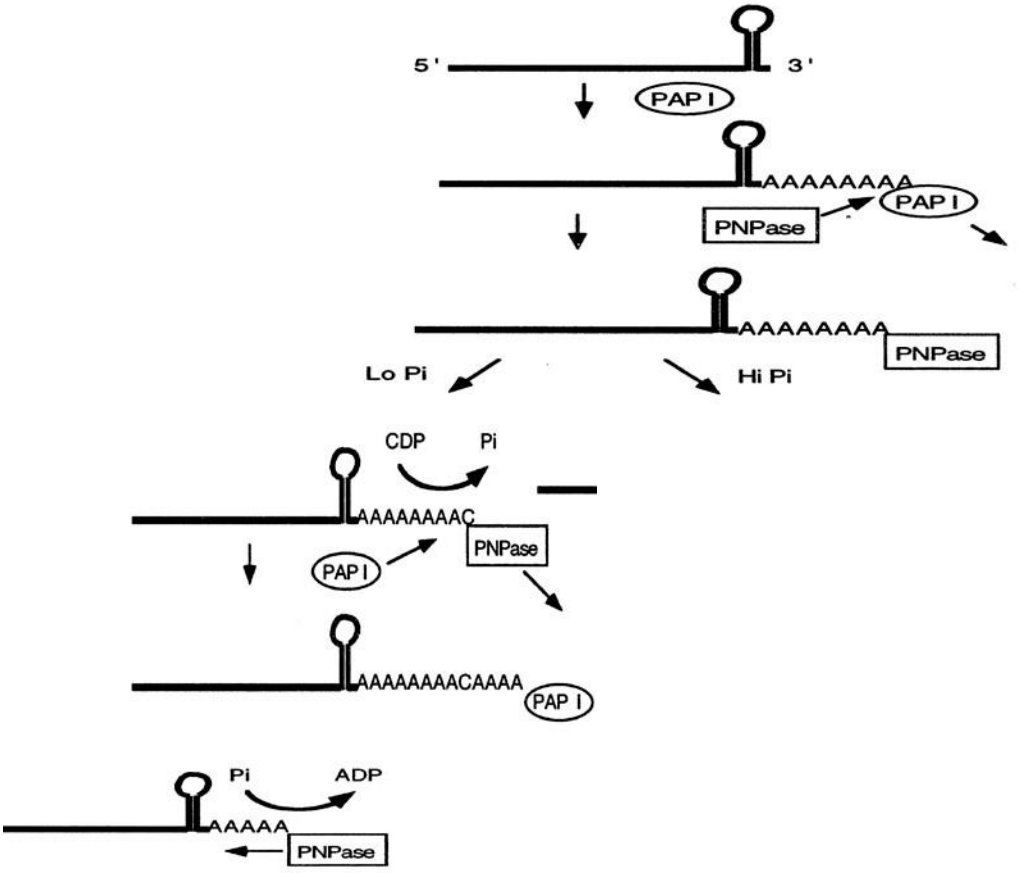

Fig: Schematic representation of the role of PNPase in poly(A) tail metabolism in *E. coli*.

In *E.coli*, polynucleotide phosphorylase regulates mRNA processing either by adding ribonucleotides to the 3' end or by cleaving bases in 3' to 5' direction. The function of PNPase depends upon inorganic phosphate (Pi) concentration inside the cell. The transcripts are polyadenylated using enzyme polyadenylate polymerase I (PAPI).

After primary polyadenylation of the transcript by PAP I, PNPase may bind to the 3′ end of the poly(A) tail. PNPase works either degradatively or biosynthetically inside the cell depending on the Pi concentration. Under high Pi concentration, it degrades the poly(A) tail releasing adenine diphosphates. If the Pi concentration is low, PAP I initiates addition of one or more

nucleotides to the existing poly (A) tail and in the process generates inorganic phosphate. On dissociation of PNPase, the 3′ end again is available to PAP I for further polymerization.

Function:

Different functions of Polynucleotide phosphorylase are:

- It is involved in mRNA processing and degradation in bacteria, plants, and in humans.
- It synthesizes long, highly heteropolymeric tails *in vivo* as well as accounts for all the observed residual polyadenylation in poly(A) polymerase I deficient strains.
- PNPase function as a part of **RNA degradosome** in *E.coli* cell. RNA degradosome is a multicomponent enzyme complex that includes RNaseE (endoribinuclease), polynucleotide phosphorylase (3’ to 5’ exonuclease), RhlB helicase (a DEAD box helicase) and a glycolytic enzyme enolase. This complex catalyzes 3’ to 5’ exonuclease activity in presence of ATP. In eukaryotes, the exosomes are located in nucleus and cytoplasm. Degradsomes in bacteria and exosomes in eukaryotes are associated with processing, control, and turnover of RNA transcripts.
- In rDNA cloning technology, it has been used to synthesize radiolabelled.

  polyribonucleotides from nucleoside diphosphate monomers.

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Deoxyribonuclease (DNase):

- A nuclease enzyme that can catalyze the hydrolytic cleavage of phosphodiester bonds in the DNA backbone are known as deoxyribonuclease (DNase).
- Based on the position of action, these enzymes are broadly classified as endodeoxyribonuclease (cleave DNA sequence internally) and exodeoxyribonuclease (cleave the terminal nucleotides).
- Unlike restriction enzymes, DNase does not have any specific recognition/restriction site and cleave DNA sequence at random locations.
- There is a wide variety of deoxyribonucleases known which have different substrate specificities, chemical mechanisms, and biological functions. They are:

1) Deoxyribonuclease I (DNaseI):

An endonuclease which cleaves double-stranded DNA or single stranded DNA. The cleavage preferentially occurs adjacent to pyrimidine (C or T) residues. The major products are 5'-phosphorylated bi-, tri- and tetranucleotides. It requires divalent ions (Ca^{2+} and Mn^{2+}/Mg^{2+}) for its activity and creates blunt ends or 1-2 overhang sequences.

DNaseI is the most widely used enzyme in cloning experiments to remove DNA contamination from mRNA preparation (to be used for cDNA library preparation, northern hybridization, RT-PCR etc). The mode of action of DNaseI varies according to the divalent cation used.

In the presence of magnesium ions (Mg^{+2}), DNaseI hydrolyzes each strand of duplex DNA producing single stranded nicks in the DNA backbone, generating various random cleavages.

On the other hand, in the presence of manganese ions (Mn^{+2}), DNaseI cleaves both strands of a double stranded DNA at approximately the same site, producing blunt ended DNA fragments or with 1-2 base overhangs. The two major DNases found in metazoans are: deoxyribonuclease I and deoxyribonuclease II.

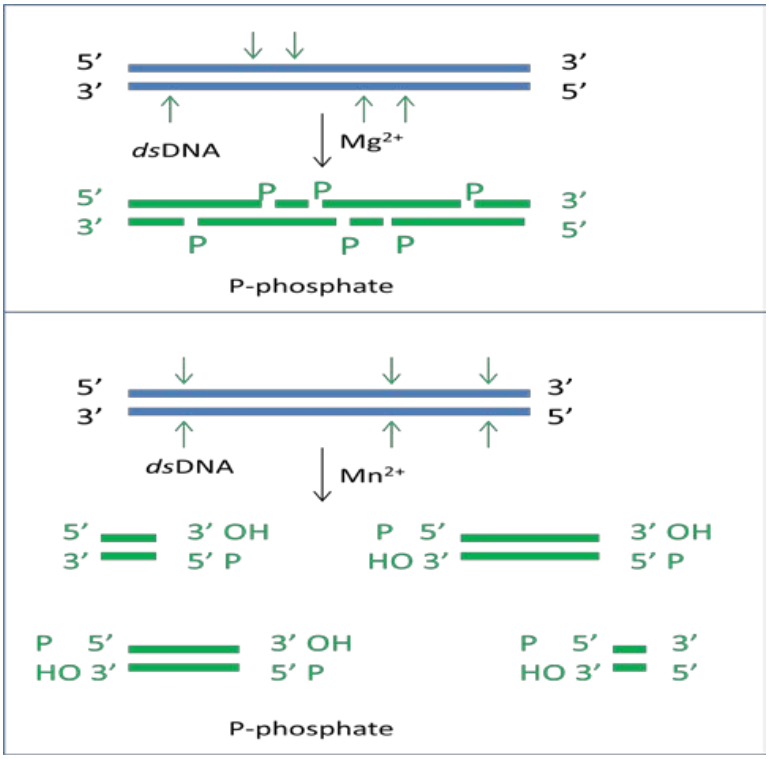

Fig : Action of DNase I in the presence of Mg^{+2} and Mn^{+2} ions. (Arrowhead denoting random site of cleavage in double stranded DNA by DNase I)

Some of the common applications of DNase I in rDNA technology have been mentioned below:

- Eliminating DNA contamination (e.g. plasmid) from preparations of RNA.
- Analyzing the DNA-protein interactions via DNA foot printing.
- Nicking DNA prior to radio-labeling by nick translation.

2) DeoxyribonucleaseII (DNaseII):

It is a non-specific endonuclease with optimal activity at acidic pH (4.5-5.5) and conserved from human to *C.elegans*.. It does not require any divalent cation for its activity. DNaseII initially introduces multiple single stranded nicks in DNA backbone and finally generates 3' phosphate groups by hydrolyzing phosphodiester linkages.

This enzyme releases 3'phosphate groups by hydrolyzing phosphodiester linkage and creating nicks in the DNA backbone. DNaseII acts by generating multiple single stranded nicks followed by production of acid soluble nucleotides and oligonucleotides.

The catalytic site of the enzyme contains three histidine residues which are essential for enzyme activity.

Some of the common applications of DNase II are as follows:

- DNA fragmentation
- Molecular weight marker • Cell apoptosis assays etc.

3)Exonuclease III:

Exonuclease III is a globular enzyme which has 3'→5' exonuclease activity in a double stranded DNA. The template DNA should be double stranded and the enzyme does not cleave single stranded DNA. The enzyme shows optimal activity with blunt ended sequences or sequences with 5' overhang.

Exonuclease III enzyme has a bound divalent cation which is essential for enzyme activity. The mechanism of the enzyme can be affected by variation in temperature, monovalent ion concentration in the reaction buffer, and

structure and concentration of 3'termini. The enzyme shows optimal activity at 37°C at pH 8.0.

Various application of exonuclease III in molecular cloning experiments are:

- To generate template for DNA sequencing
- To generate substrate for DNA labeling experiments
- Directed mutagenesis.
- DNA-protein interaction assays (to find blockage of exonuclease III activity by protein-DNA binding) etc.

4) Mung bean nuclease:

As the name suggest, this nuclease enzyme is isolated from mung bean sprouts (*Vigna radiata*). Mung bean nuclease enzymes can degrade single stranded DNA as well RNA. Under high enzyme concentration, they can degrade double stranded DNA, RNA or even DNA/RNA hybrids.

Mung bean nuclease can cleave single stranded DNA or RNA to produce 5'-phosphoryl mono and oligonucleotides. It requires Zn^{2+} ion for its activity and shows optimal activity at 37°C. The enzyme works in low salt concentration (25mM ammonium acetate) and acidic pH (pH 5.0). Treatment with EDTA or SDS results in irreversible inactivation of the enzyme.

Mung bean nuclease is less robust than S1 nuclease and easier to handle. It has been used to create blunt end DNA by cleaving protruding ends from 5' ends. This enzyme cannot produce nicks in a double stranded DNA but at higher concentration, it can generate nicks and cleave double stranded DNA.