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ENZYMES IN MODIFICATION
PHOSPHATASES AND METHYLASES
AND THEIR MECHANISM OF ACTION

Phosphatase:

- Phosphatase catalyses the cleavage of a phosphate (PO_4^{-2}) group from substrate by using a water molecule (hydrolytic cleavage).
- This reaction is not reversible.
- This shows totally opposite activity from enzyme like kinase and phosphorylase that add a phosphate group to their substrate. On the basis of their activity there are two types of phosphatases i.e acid phosphatase and alkaline phosphatase. In both forms the alkaline phosphatase are most common.
- Special class of phosphatase that remove a phosphate group from protein, called "Phosphoprotein phosphatase".

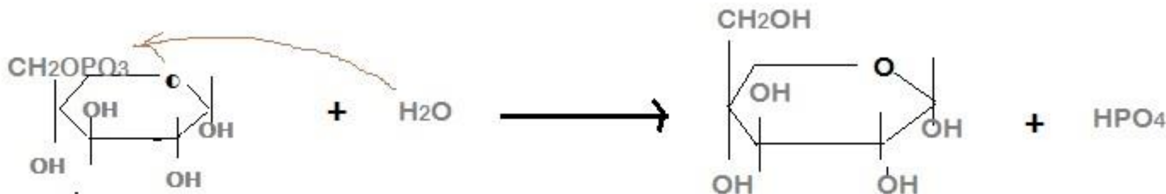

Fig: Schematic representation of hydrolytic cleavage of phosphate group ($-PO_4^{-2}$).

1. Acid phosphatase:

- It shows its optimal activity at pH between 3 and 6, e.g. a lysosomal enzyme that hydrolyze organic phosphates liberating one or more phosphate groups. They are found in prostatic epithelial cells, erythrocyte, prostatic tissue, spleen, kidney etc.
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2. Alkaline phosphatase:

- Homodimeric enzyme which catalyzes reactions like hydrolysis and transphosphophorylation of phosphate monoester.
- They show their optimal activity at pH of about 10.
- Alkaline phosphatase was the first zinc enzyme discovered having three closed spaced metal ion. Two Zn^{+2} ions and one Mg^{+2} ion, in which Zn^{+2} ions are bridges by *Asp* 51. The mechanism of action is based on reaction where a covalent serine – phosphate intermediate is formed to produce inorganic phosphate and an alcohol.
- In human body it is present in four isoforms, in which three are tissue specific isoform i.e. placental, germ cell, intestinal and one is non tissue specific isoform. The genes that encode for tissue specific isoforms are present on chromosome -2 p37-q37, while the genes for one non tissue specific are present on chromosome 1 p34- p36.1.
- During post-translational modification, alkaline phosphatase is modified by Nglycosylation. It undergoes a modification through which uptake of two Zn^{+2} ion and one Mg^{+2} ion occurs which is important in forming active site of that enzyme. Alkaline phosphatases are isolated from various sources like microorganisms, tissue of different organs, connective tissue of invertebrate and vertebrate, and human body.

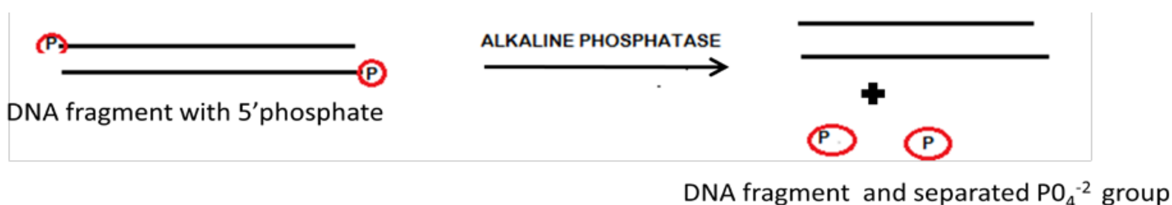

Fig: Action of alkaline phosphatase

There are several AP that are used in gene manipulation-

- **Bacterial alkaline phosphatase (BAP) -** Bacterial alkaline phosphate is a phosphomonoester that hydrolyzes 3' and 5' phosphate from nucleic acid (DNA/ RNA). It more suitably removes phosphate group before end labeling and remove phosphate from vector prior to insert ligation. BAP generally shows optimum activity at temperature 65°C. BAP is sensitive to inorganic phosphate so in presence of inorganic phosphates activity may reduce.
- **Calf intestinal alkaline phosphatase (CIP) –** It is isolated from calf intestine, which catalyzes the removal of phosphate group from 5' end of DNA as well as RNA. This enzyme is highly used in gene cloning experiments, as to make a construct that could not undergo self-ligation. Hence after the treatment with CIP, without having a phosphate group at 5' ends a vector cannot self-ligate and recircularise. This step improves the efficiency of vector containing desired insert.
- **Shrimp alkaline phosphatase (SAP) -** Shrimp alkaline phosphatase is highly specific, heat labile phosphatase enzyme isolated from arctic shrimp (*Pandalus borealis*). It removes 5' phosphate group from DNA, RNA, dNTPs and proteins. SAP has similar specificity as CIP but unlike CIP, it can be irreversibly inactivated by heat treatment at 65°C for 15mins. SAP is used for 5' dephosphorylation during cloning experiments for various application as follows:
  - Dephosphorylate 5'-phosphate group of DNA/RNA for subsequent labeling of the ends.
  - To prevent self-ligation of the linearized plasmid.

- To prepare PCR product for sequencing.
- To inactivate remaining dNTPs from PCR product (for downstream sequencing appication).

Two primary uses for alkaline phosphatase in DNA modification:

- Removing 5' phosphate from different vector like plasmid, bacteriophage after treating with restriction enzyme. This treatment prevents self-ligation because unavailability of phosphate group at end. So, this treatment greatly enhances the ligation of desired insert. During ligation of desired insert, the complementary ends of the insert and vector will come to proximity of each other (only for sticky ends but not for blunt ends). One strand of the insert having 5'-phosphate will ligate with the 3'OH of the vector and the remaining strand will have a nick. This nick will be sealed in the next step by ligase enzyme in the presence of ATP. It is used to remove 5' phosphate from fragment of DNA prior to labeling with radioactive phosphate.

3.Methylase:

- Methyltransferase or methylase catalyzes the transfer of methyl group (-CH_3) to its substrate. The process of transfer of methyl group to its substrate is called methylation.
- Methylation is a common phenomenon in DNA and protein structure.
- Methyltransferase uses a reactive methyl group that is bound to sulfur in Sadenosyl methionine (SAM) which acts as the methyl donor.

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- Methylation normally occurs on cytosine (C) residue in DNA sequence. In protein, methylation occurs on nitrogen atom either on N-terminus or on the side chain of protein.
- DNA methylation regulates gene or silence gene without changing DNA sequences, as a part of epigenetic regulation.
- In bacterial system, methylation plays a major role in preventing their genome from degradation by restriction enzymes. It is a part of restriction – modification system in bacteria.

Methyltransferase can be classified in three groups:

a) m6A-generates N6 methyladenosine,

b) m4C-generates N4 methylcytosine,

c) m5C-generatesN5 methylcytosine.

m6A and m4C methyltransferase are primarily found in prokaryotes. These enzymes are responsible for methylation of DNA sequences in order to prevent the host from digesting its own genome via its restriction enzyme.

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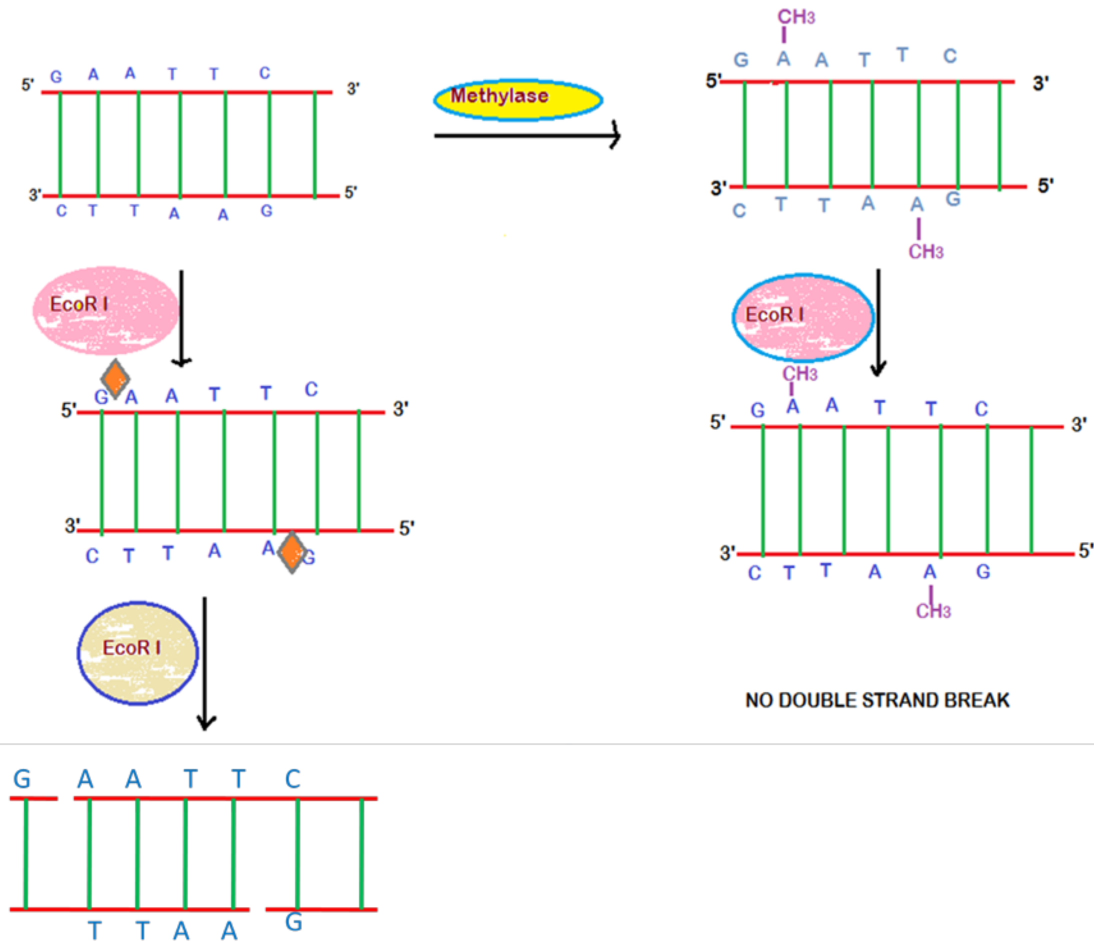

Fig: Activity of restriction and methylase enzymes

Restriction enzyme *Eco*RI cleaves within the recognition sequence if the DNA is unmethylated. On methylation by methylases, the restriction enzyme *Eco*RI is inhibited from cleaving within the restriction site.

Some common examples of methytransferases are DNA adenyl methytransferase (DAM), histone methyltransferase, O-methyltransferase etc. DAM methylase is generally used in recombinant DNA technology which can methylate adenine (A) in the sequence 5'GATC3'. This enzyme can methylate a newly synthesized DNA strand on specific sites.