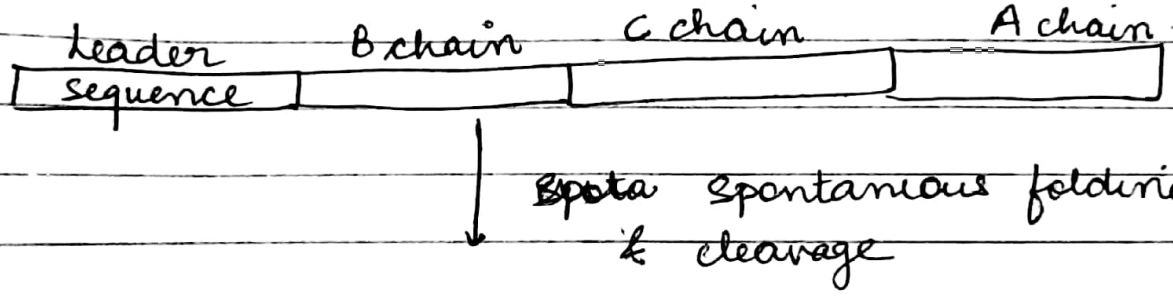
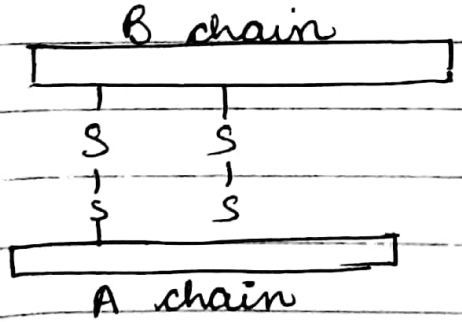
Recombinant Insulin

- it can be recombinantly expressed in bacteria as no post translation modification required.
- Also the size of insulin is small:- insulin contains 2 chains A chain - 21 a.a. long, B chain 30 aa. long joined by disulfide bridges.

In humans, the insulin is synthesized as Preproinsulin which is processed further to form active insulin

Preproinsulin has a





Insulin (Active)

Synthesis

- Appropriate expression vector is chosen.
- DNA sequence of A gene and B gene is cloned separately

- Vectors are transformed and expressed with IPTG induction
- A chain Polypeptide & B chain polypeptides are purified by affinity chromatography.
- Purified A and B chains are mixed together and allowed to form disulfide bridges.

Recombinant factor VIII

- factor VIII → role in blood clotting
- 186Kb in length in DNA, split it into 26 exons mRNA codes for 2351 a.a., also requires post Translation modifications. Hence can't be expressed in E. Coli.
- Therefore expressed in hamster cells.

Refer to Gene cloning by TA Brown edition 7

chapter - 14 Applications of Gene cloning & DNA analysis in Biotechnology.

Transgenic crops

→ Insecticide resistant 'Bt' crops

↳ Bacillus thuringiensis - part of insect diet - evolved a defense mechanism against insect predation.

↳ While sporulating, they code for protein δ-endotoxin which is insecticidal.

↳ δ endotoxin is produced as inactive precursor. After ingestion, modifications like cleavage produces active form which display toxicity. This toxin binds to gut epithelium of insects and damage it.

These proteins are charecterized by their insecticidal activity and classified into four groups -

(i) cry I - crystalline, lepidoptera specific
(ii) cry II - crystalline, lepidoptera & diptera specific
(iii) cry III - " coleoptera specific
(iv) cry IV - " diptera specific

generally Bt cry I and cry II genes are used.

For the development of any transgenic crops, certain steps are required :- ① Identification of gene and its cloning
② cloning of the gene in Agrobacterium Ti plasmid based vector
③ Transfer of Ti plasmid based vector into plants tissue like protoplast / callus.

generally cry1Ac and cry1Ab genes are used.

(4) Recovery of transformed tissue

Gene transfer in protoplast by Biolistic technique is also useful and Agrobacterium mediated transfers were initially used.

Benefits of Bt transgenics – Reduction in the usage of insecticides
- ✓ reduce the potential collateral damage to non target species
- ✓ improved yields
- ✓ Able to grow even in areas with high insect infestation
- ✓ reduce environmental pollution & health hazards related to use of chemicals.

Herbicide Resistance

Glyphosate also known as Round up by Monsanto Corporation is important herbicide which inhibit the enzyme – 5-enolpyruvyl shikimate-3-PO_4 synthase (EPSPS) of shikimate pathway. Resistance to herbicide was developed by putting EPSPS-gene from glyphosate resistant strain of E.Coli under plant promoter. Such transgenic crops are known as "Roundup Ready".

~~A glyphosate~~

A glyphosate tolerant cell line from Petunia hybrida was observed to overexpress EPSPS as a result of gene amplification. The gene from this plant was cloned under strong plant promoter like CaMV.
EPSPS was overexpressed in chloroplast and plants found to be tolerant to herbicides.

Another important herbicide is Phosphinothricin (PPT)
It is irreversible inhibitor of plant glutamine synthetase.
Certain bacteria produce enzyme phosphinothricin acetyltransferase (PAT), which inactivate PPT by acetylation. The gene for PAT is known as 'bar' that has been introduced in several crops using Agrobacterium mediated transformation.

Benefits:- (1) increased yield
(2) Better seed quality as competing weeds are eliminated
(3) Less environmental pollution

Principles of Gene Manipulation by Primrose et al 7th Edition

Molecular Biotechnology by Glick et al 4th Edition

Ref. Molecular Biotechnology - Glick et al 4th Edition

Chemical Synthesis of DNA

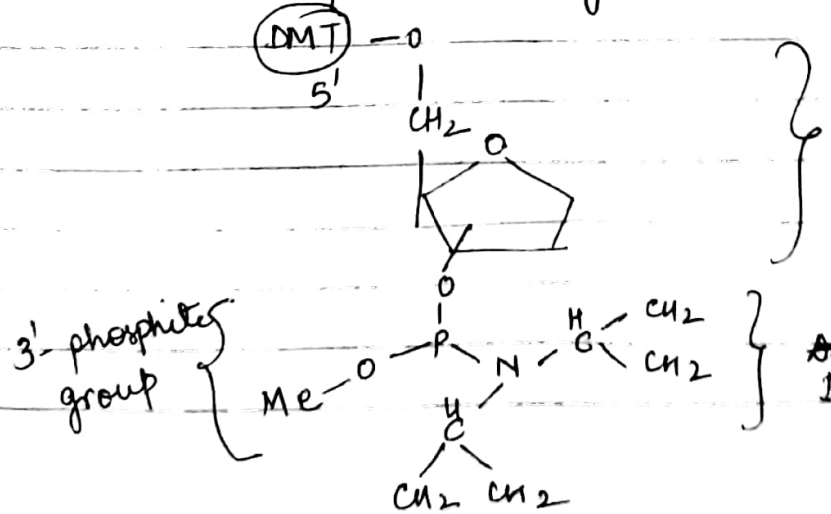
Phosphoramidite Method

- Synthesis take place from 3' → 5'
incoming nucleotide is coupled to the 5'-OH terminus of growing chain.

→ Nitrogenous Bases A, G & C are derivatised by additions of Benzoyl, isobutryl groups at the $-NH_2$ groups of the bases to prevent undesirable site reactions.
T can't be treated because they lack amino group.

→ First nucleotide would be the base at 3' end.
is attached to Spacer molecule which is attached to inert Support (controlled Pore glass CPG)

→ DMT group (dimethoxytrityl) is at 5' end of nt protecting the 5'-end.
3'-end is protected by phosphite group.



Structure of Phosphoramidite ⇓ incoming 'Nt'

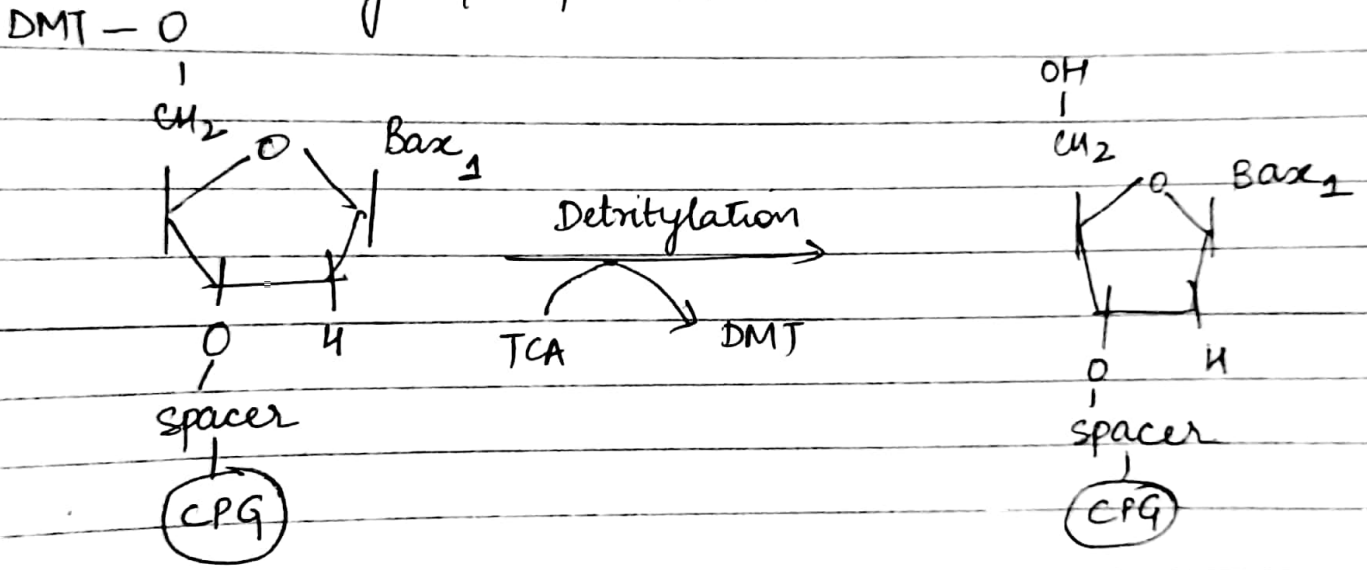
3'-phosphite group

Diisopropylamine group

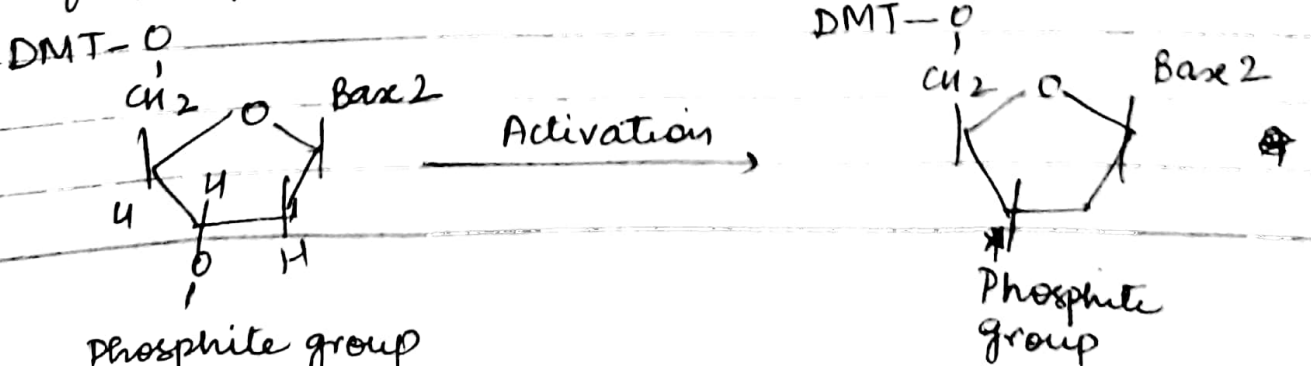
Steps in chemical synthesis in DNA

(1) linking the first nucleotide to the column.
3' nt at 3'- end would be the first nucleotide which would be attached to CPG.

(2) ~~Detry~~ Detritylation :- Removal of 5' DMT group from ~~the~~ ~~phosphoramidite~~ the nucleotide attached to CPG

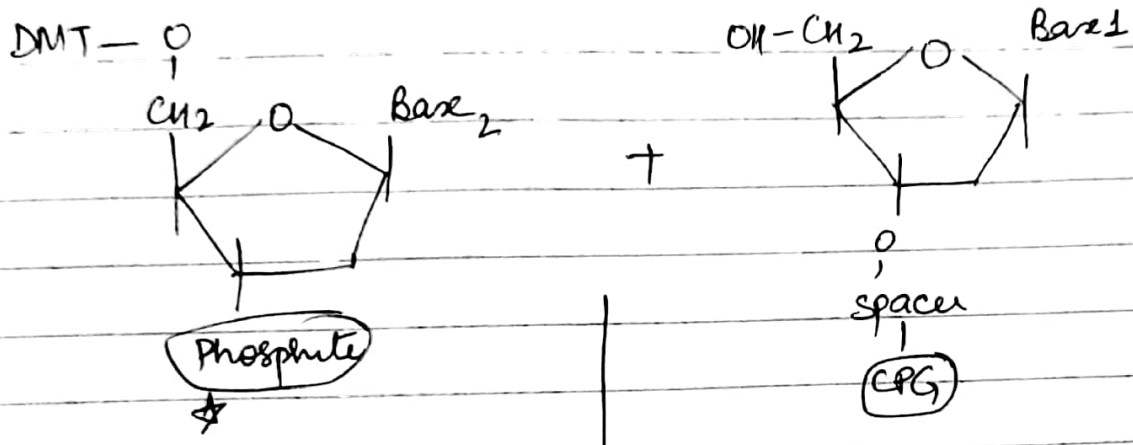


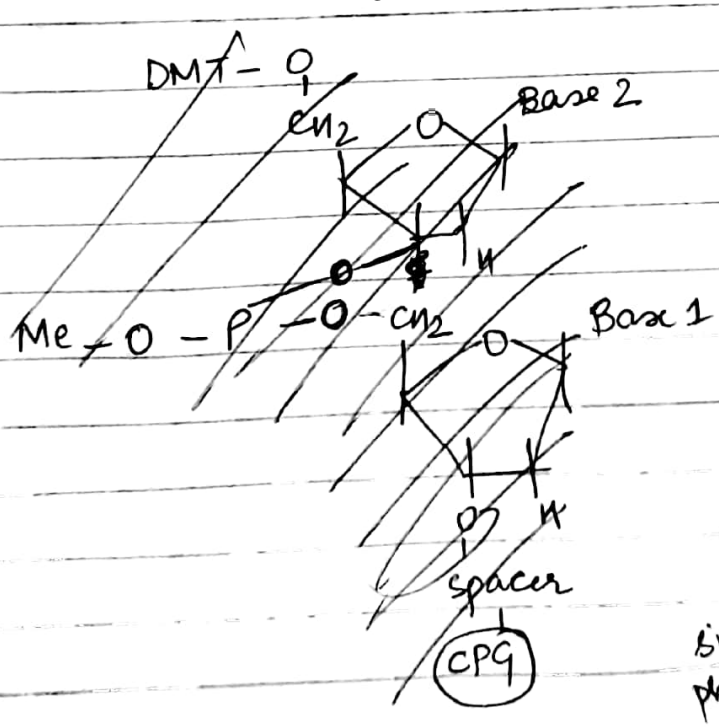
(3) Activation and coupling
2-nt and further elongation is done by addition of phosphoramidite.

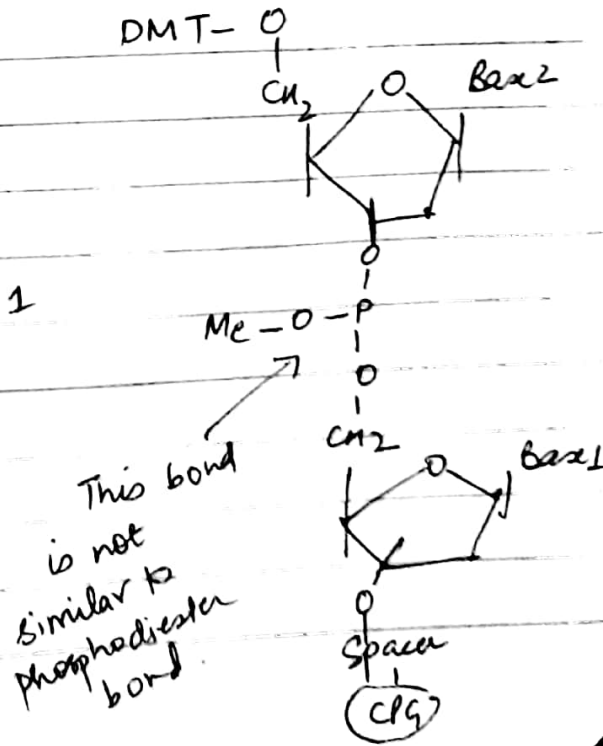


This activation will cause the unstable 3'- phosphite end to get bonded with 5'- end of Base-1.

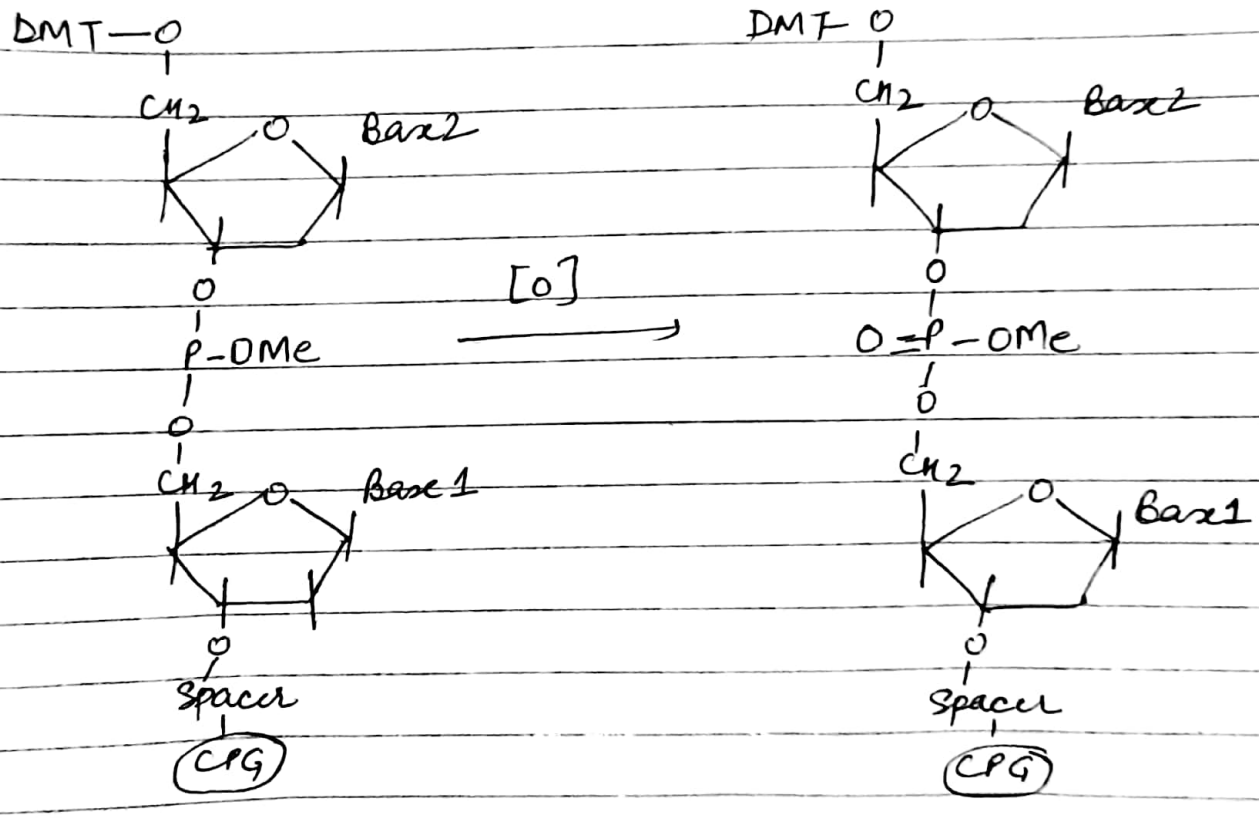
(4) Coupling



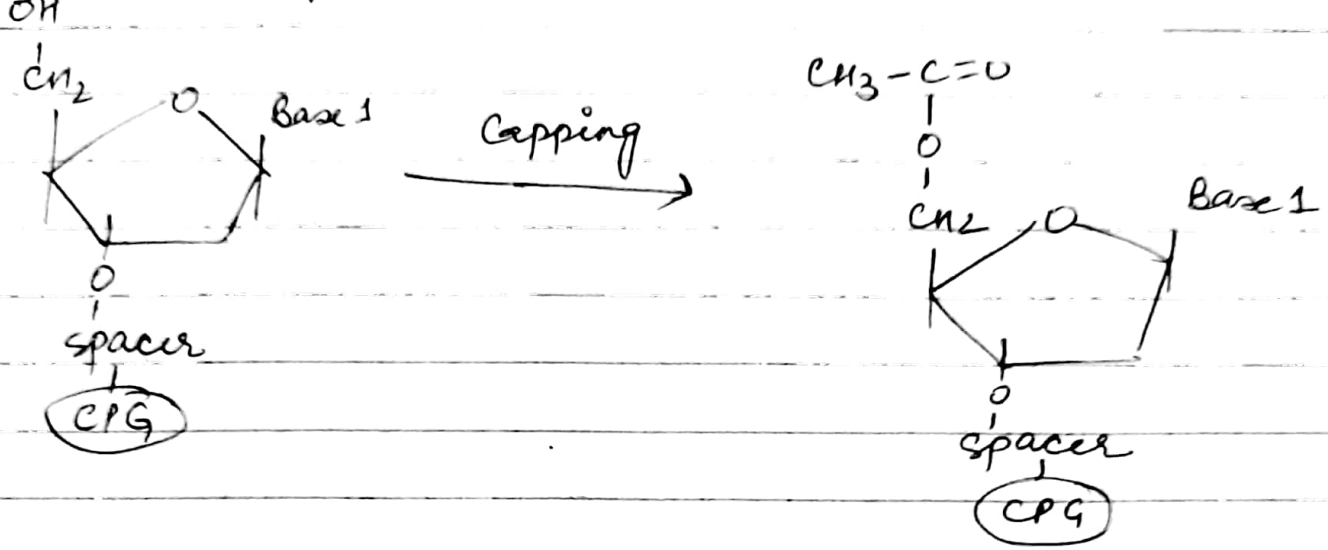




(5) Oxidation :- The phosphite triester internucleotide linkage is oxidised to the pentavalent phosphate bond. This reaction stabilizes the phosphodiester bond & makes it less susceptible to cleavage.



(6) Capping:- The nucleotides which remain unused, there 5' ends are protected by acetylation to prevent coupling unnecessarily



Uses:- primer synthesis
gene synthesis