- **Nucleophilic attack occurs on the 3'OH of the primer by the incoming alpha phosphate of the incoming nucleotide with a PPi being expelled therefore polymerisation occurs in the 5' to 3' direction. This is a universal rule of polymerases.**
- Fidelity maintenance:
    - Have an intrinisic 3' to 5' exonuclease which can remove nucleotides which have been incorrectly incorperated
    - When error is detected by polymerase this exonuclease is activated.
- To ensure processivity (continuous sleek unidirectional replication) ring shaped **B subunint clamp** protein holds DNA polymerase onto the template DNA which is more efficient.

- Evidence to detect activity of the polymerase:
    - using radioactive TTP to monitor DNA synthesis as it is only used by DNA and not RNA.
    - If it was a double stranded DNA it would not have worked but because he used a biochemical assay there were gaps which the polymerase was able to fill.
    - Isolated from E coli as it grows rapidly and can be harvested in large quantities and purify DNA using

Prokaryote DNA replication (E.coli) :

- Prokaryotes have a single replication of origin called **OriC.**

- First **Recognition** of OriC and **separation** of DNA strand at replication of origin.

- This is achieved by DnaA which is an initiator protein that binds to the **dnaA box** region present in OriC to form the orisome complex which causes the DNA in this region to **negatively supercoil**.

- Negative supercoiling of dnaA box causes separation of **dnaB box** region **downstream which** puts torque on the nearby AT-rich region to denature and form a **replication bubble**.

- DnaA complex recruits **DnaB (helicase)** on opposite sides of separated DNA strands and expands the replication bubble in 5'to 3' direction using energy from ATP hydrolysis moving bidirectionally in opposite directions.

- Breaking of hydrogen bonds by DnaB introduces positive supercoils infront of DnaB therefore **DNA gyrase** or **topoisomerases** bind to DNA ahead to counter this stress.

- **SSBPs (single strand binding proteins**) also bind to prevent reannealing of strands or formation of another structure which would block replication.

- **DnaG** (**primase** - type of RNA polyermase) synthesises an RNA primer required for DNA polymerases to work as they can only extend not start chain. Primase forms **primosome** complex with template with DNA and additional proteins.

- Main polymerase responsible for most of elongation in prokaryotes is **DNA polymerase III** consists of **holoenzyme** complex contains core subunits:

    - Alpha – responsible for majority of DNA synthesis
    - Epsilon – proof reading activity in this subunit aslong as bound to alpha subunit and greater mutations in strains without this subunit
    - Thetha
    - Beta - forms a ring like clamp to hold in place and ensure processivity and must be loaded onto DNA by clamp loader which requires energy from ATP hydrolysis

- **DNA poly I** first to be identified;
    - role is for **REPAIR**

- Extensively cytosine methylated **CpG dinucleotides** (these normally mark active genes) by MeCP2 proteins
- Enriched histone varients
- **H3 and H4 hypoacetylated**
- methylation = silence mechanism
    - **TFs** cant bind methylated DNA
    - Changes the way **H1 binds to DNA**
- Eg in tortoise shell cats inactivation spreads along the chromosne from a discrete nucleation site and is reactivated during germ cell formation

EXTRA not directly related to histones:

1. Subnuclear localisation of genes:
    a. Genes located near to the nuclear periphery are less likely to be expressed so silencing genes may be positioned there

Evidence:

- Cleavage by micrococcol nuclease is preferential where linker DNA is present showing presence of nucleosomes
- Show nucleosomes involved in transcription: genes are **more sensitive to nuclease** digeston in **tissues in which they are transcribed.**

These studies cannot distinguish between cause and effect of active transcription whereas:

- **Genetic studies of histone mutations**
- **In vivo transcription systems**

Prok regulation

- Genetic switch transcription factors:
    - DNA binding proteins distort the structure of the DNA to cause bending
    - Simplest structure contains helix turn helix motif which is 2 helices at a fixed angle:
        - Trp repressor in prok
- When tryptophan acts as an **activator of the repressor** as if in medium no point making it
- **2 tryptophan** molecules bind to repressor and tilt the helix turn helix motif of the repressor so it can bind to **the major groove**.
- Repressor then competes with RNAP so it cannot transcribe mRNA.

        - Homeodomain in euk
    - Measure DNA and protein interaction by radioactively labelled DNA and doing Gel electrophoresis. Protein will be radioactive if bound.

Enhancers

1. Allow for fine tuning
2. Region of DNA where activators will bind to form a loop in the DNA contacting the promoter which will recruit the **mediator multiprotein complex**:
    - Recruits Transcription factors which are normally diffusion limited
    - Modulates phosphorylation of serines of YSPTSPS

Silencers

- Block binding of activators to enhancers
- Make chromatin more compact and less accessible

Example: Lac operon in E.coli – negative regulatory control

This mechanism saves energy as the enzymes B-galactosidase and lactose permase are only synthesized when lactose is present in the cell.

1. Multiple AUG start codons on mRNA so multiple potential reading frames

2. 10 bps upstream of desired AUG codon is **Shine Dalgerno sequence (AGGA)** which is **complementary** to the seqeuence of the **16S rRNA**

3. AUG codes for methionine normally but first start AUG codon codes for formylated methionine (fMet), formylation:
    a. Blocks free amino acid group thus preventing the initiator from participating in the polypeptide elongation
    b. Increases efficiency of IF2 to fMet-tRNA

4. tRNA brings AA to ribsome:
    a. 2ndry cloverleaf structure with three stem loops
        i. variable region
        ii. acceptor stem where AA is bound (CAA sequence – binds to 2'OH of adenosine in nucleophilic reaction)
        iii. anticodon = 3' single stranded region
    b. no base pairing in loops as they have modifications to their bases
    c. 3ry structure = L shape

5. **Amino acyl synthase enzymes:** transfer amino acid to appropriate tRNA molecule and there is a specific enzyme for each whose active site is complementary in terms of:
    a. Shape
    b. charge
    c. size

6. 2 step reaction which is longer so allows time for incorrect AA to diffuse away:
    a. Hydrolysis of ATP molecule to produce PPi and **Amino acid-AMP complex**
    b. Amino acid then added to tRNA and AMP is released

7. Assembley of the preinitiation complex:
    a. **IF1** – blocks A site
    b. **IF3** blocks E site and maintains dissociation of subunits
        i. These two binding allows fMet-tRNA to only enter the P site in the major groove portion of the 16S rRNA
        ii. P site holds tRNA with growing polypeptide chain
        iii. Fmet- tRNA can enter P site without having to go through P site as three GC pairs preceeding stem loop so correct geometry and H bonds for P site entry.
    c. IF2 – GTP bound:
        i. Binds to fMet-tRNA and inserts tRNA into P site
        ii. Hydrolysis of GTP to GDP:
            1. Causes conformational change releasing IF3 and IF1 so large subunit can associate
            2. GTP used instead of ATP as it takes longer to hydrolyse therefore there is more time for an incorrect tRNA bound to dissociate whereas a correct one would remain bound

8. Good point of regulation: eg IF2 can be **phosphorylated** on its serine residues so it can no longer bind to fMet tRNA.

<u>Elongation</u>

-1. ribosome can only read two codons at one time so only 2 tRNAs can be present at one time

-2. **EF-Tu GTP bound:**
    a) brings in Trna into A site