REPLICATION TERMINATION

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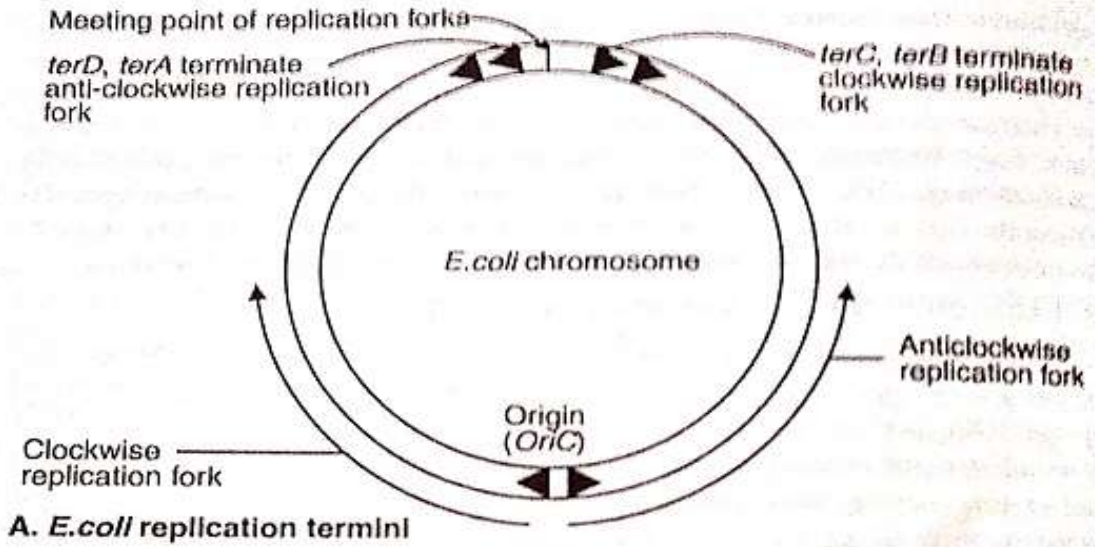
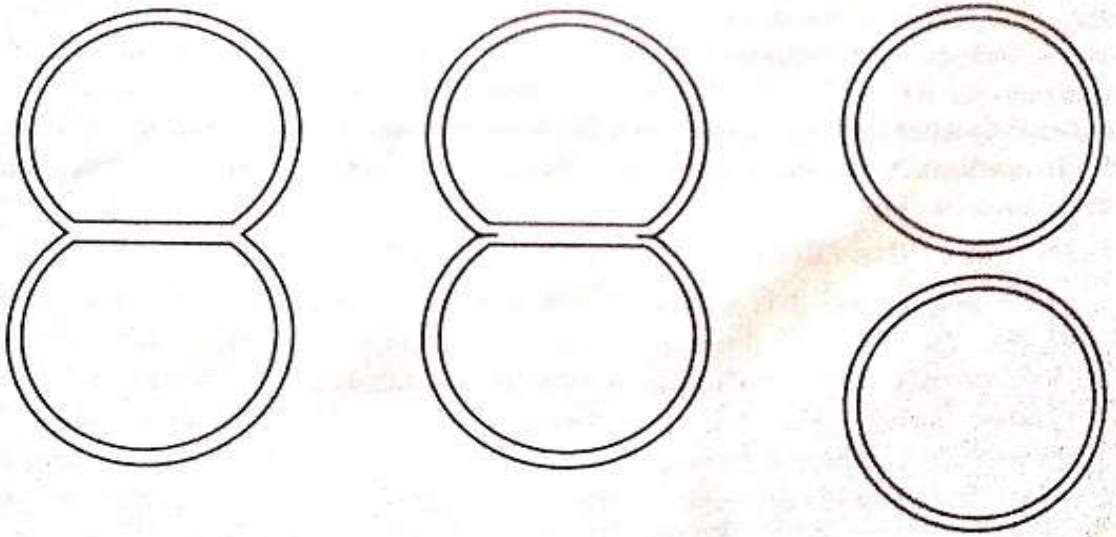
• Termination of replication occurs primarily by the meeting of the replication forks in a termination zone or ‘ter’ region.
• It produces a pair of catenated or interlocked DNA duplex circles which are then separated by a topoisomerase II (gyrase).

▪ Termination zone or ter region:
• In E.coli, the chromosome has a large termination zone or ter region located diametrically opposite, (i.e. 180^{o} around the chromosome) from the origin of replication oriC.

• During bidirectional replication the two replication forks moving in opposite (clockwise and anticlockwise) meet in the ter region.
• This region is a replication fork trap of about 450kb.
• It carries specific sequences called ter sites which stall the movement of the replication forks.
• Each ter site consists of a short (approx 23 bp) consensus sequence.
• Although the replication forks usually meet at a point midway the chromosome opposite to oriC, the termini are located beyond this point.



A. *E.coli* replication termini



Circular DNA nearing the end of θ-style replication cycle

Cateneted interlinked daughter DNA duplexes

Decatenation by topoisomerase II (gyrase)

B. Decatenation of catenated daughter DNA duplexes by gyrase

• The ter sites are arranged in an overlapping manner so that there is no replication free gap between them.
• There are two ter sites, ter D, ter A and ter C, ter B, located about 100 bp on either side of the meeting point.
• The clockwise replication fork is terminated at ter C, ter B, and the anti-clockwise fork at ter D, ter A.
• Thus the ter sites are so arranged that each replication fork would have to pass the other to reach its terminus.
• The termination region therefore functions as a replication fork trap.

• If one fork is delayed for some reason, the more rapid other fork will be trapped at the ter site and wait for the arrival of the slower fork.
• The clockwise replication fork arrives first at the terminus and is arrested.
• The anti-clockwise fork arrives somewhat later, and probably fuses with the clockwise fork.

▪ Tus-ter complexes:
• The blocking activity of the replication fork is the result of a protein called Tus (or ter-binding protein, TBP) binding to the ter sites.

• The 36 kDa Tus protein is believed to block fork movement by inhibiting the unwinding of duplex DNA catalyzed by DNA helicases.
• The Tus - ter complexes block replication fork movement in a polar manner.
• Replication forks coming to the complex from one direction are stopped, whereas those coming from the other direction proceed normally.

▪ Topological unlinking of parental DNA strands:
• Because of the helical nature of DNA the two strands are intertwined once for about every 10bp of parental DNA.

• Towards the end of replication the two daughter circular DNA duplexes are topologically interlinked.
• Such catenated interlocked circles have been observed as late replication intermediates.
• The interlinks must be removed before the replication products separate.
• It has been proposed that the decatenation of the interlocked DNA duplex circles is carried out by topoisomerases.
• In E.coli, the major decatenating enzyme is thought to be DNA topoisomerase II (gyrase).

• Type II topoisomerases catalyze topological transformation in DNA by cleaving both strands in one duplex transiently to form an enzyme-operated gate through which a segment of the other duplex is passed.

• The cut strands in the first DNA duplex are then rejoined.

➢ Mutations:

• Genome is subject to different type of heritable changes.
• A sudden and heritable change in the sequence of an organism's genome that gives rise to alternate forms of any gene is called mutation.
• It can simply be put as an abrupt change in the genotype of an organism that is not the result of recombination.
• The process by which mutations is produced is called mutagenesis.

• An organism exhibiting a novel phenotype as a result of the presence of a mutation is produced is called mutant.
• Gene mutations may be spontaneous or induced.
• Spontaneous gene mutations are those that arise in the absence of any definite cause.
• It can occur because of replication errors, spontaneous lesions and transposition of transposable elements during the normal growth of the cell.
• Induced mutations are those that are brought about experimentally by a variety of agents called mutagens.

• These include chemical mutagens and physical mutagens such as radiations and temperature shock.
• Repair of genetic lesions (mutations) keeps the harmful effects of mutations under reasonable control.

❖ Tautomerism:

The ability of a molecule to exist in more than one chemical form is called tautomerism. The spontaneous isomerization of a base to an alternative hydrogen-binding condition is called a tautomeric shift. Such shifts may result in gene mutation.

18.2

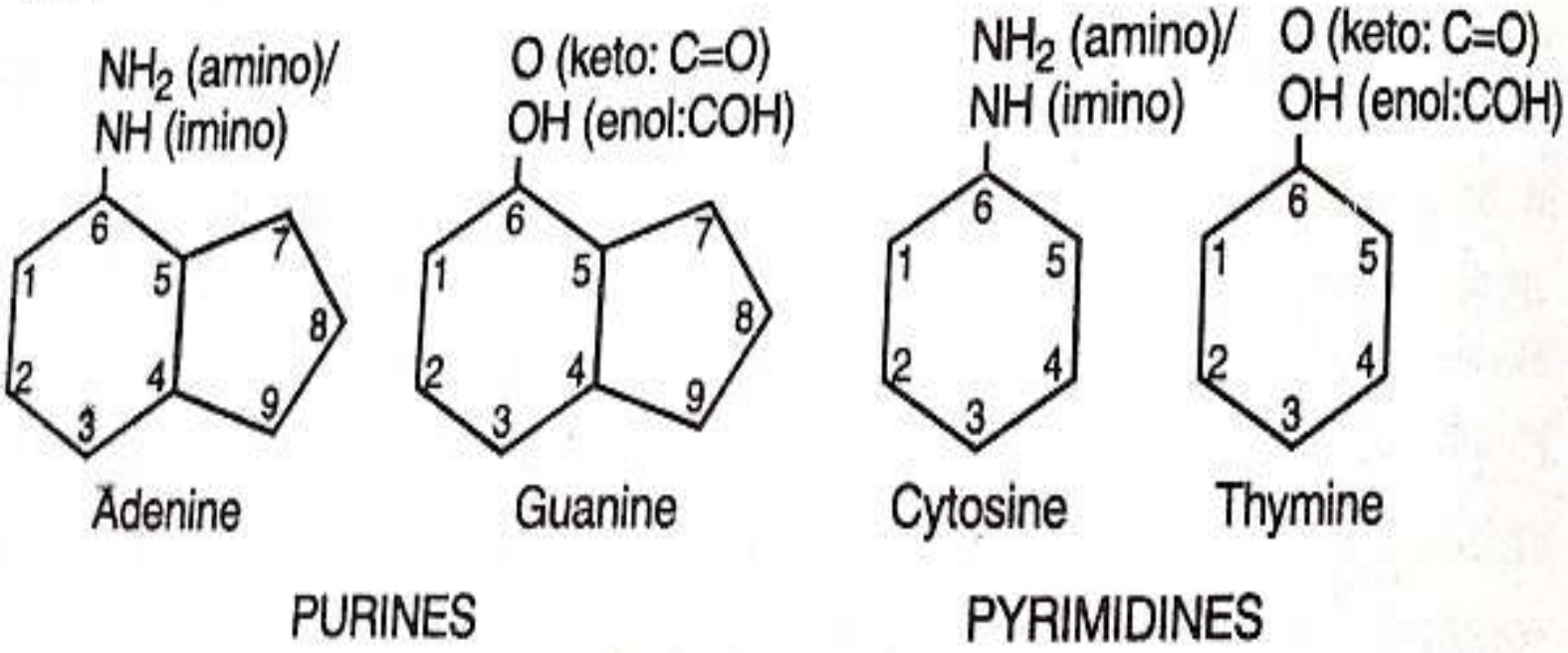


Fig. 18.1. Schematic representation of the tautomeric forms of the four DNA bases

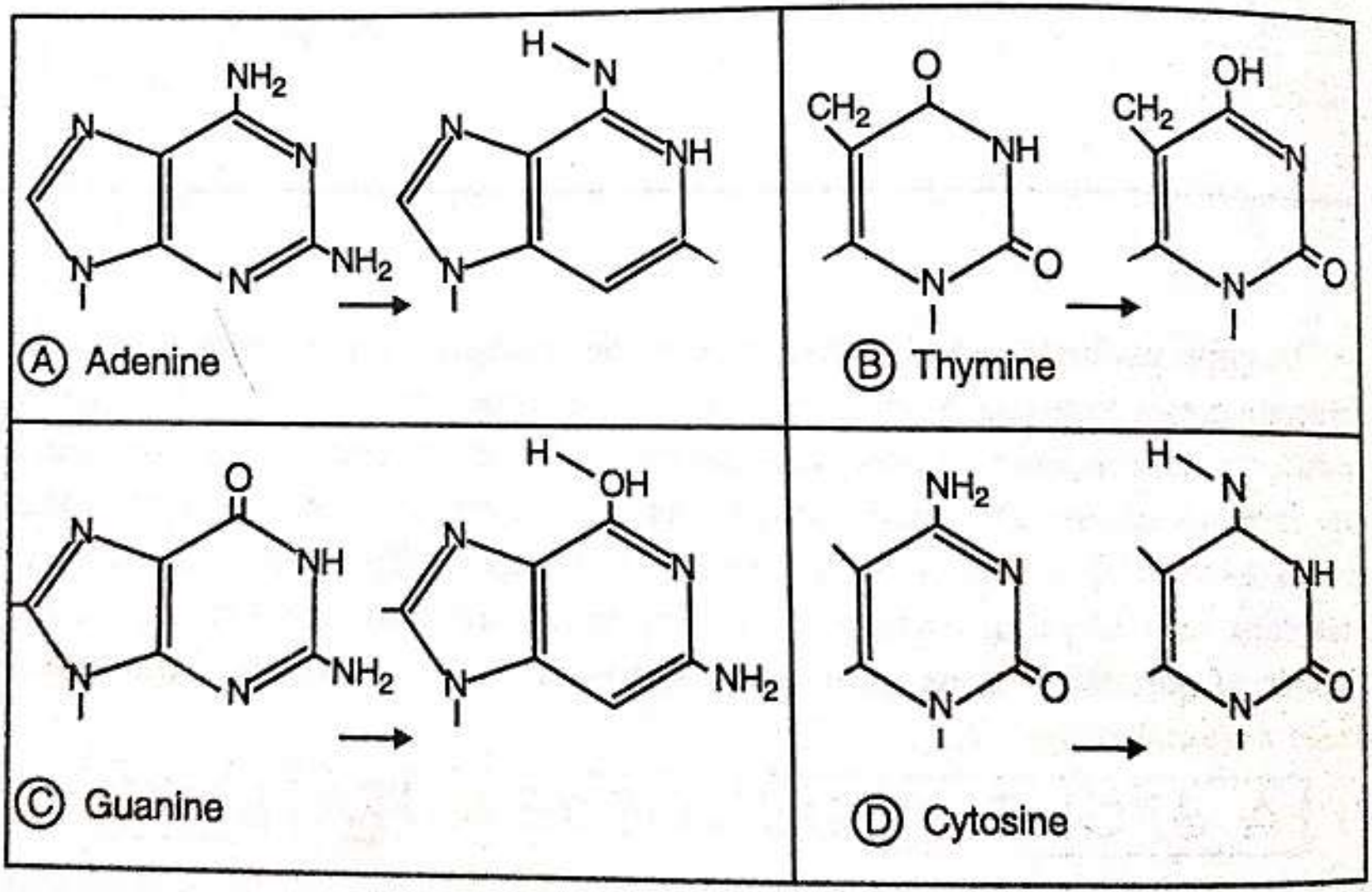


Fig. 18.2. Tautomerism of (A) adenine, (B) thymine, (C) guanine and (D) cytosine. The common state of each base is shown on the left and the rare state on the right.

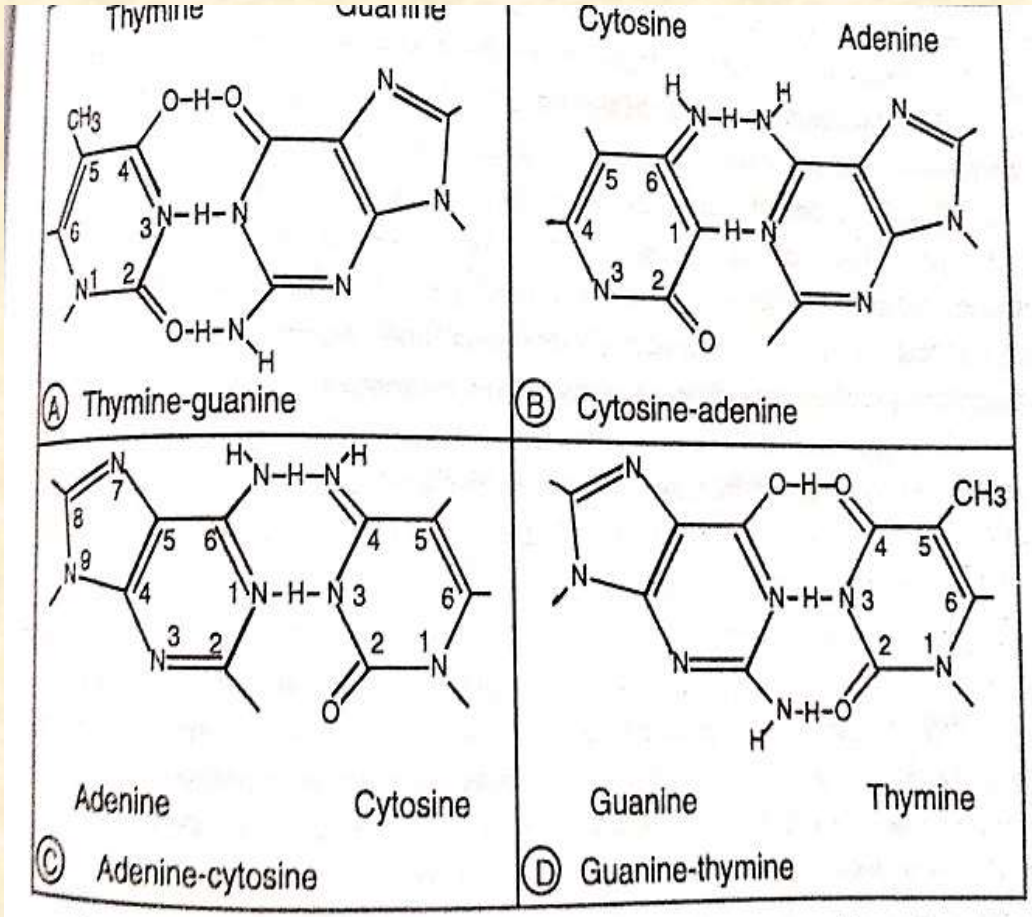


Fig. 18.4. Abnormal or forbidden base pairing resulting from tautomerism.

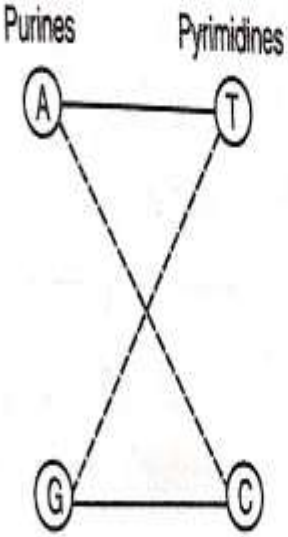


Fig. 18.3. Normal (continuous lines) and forbidden (dashed lines) base pairing.

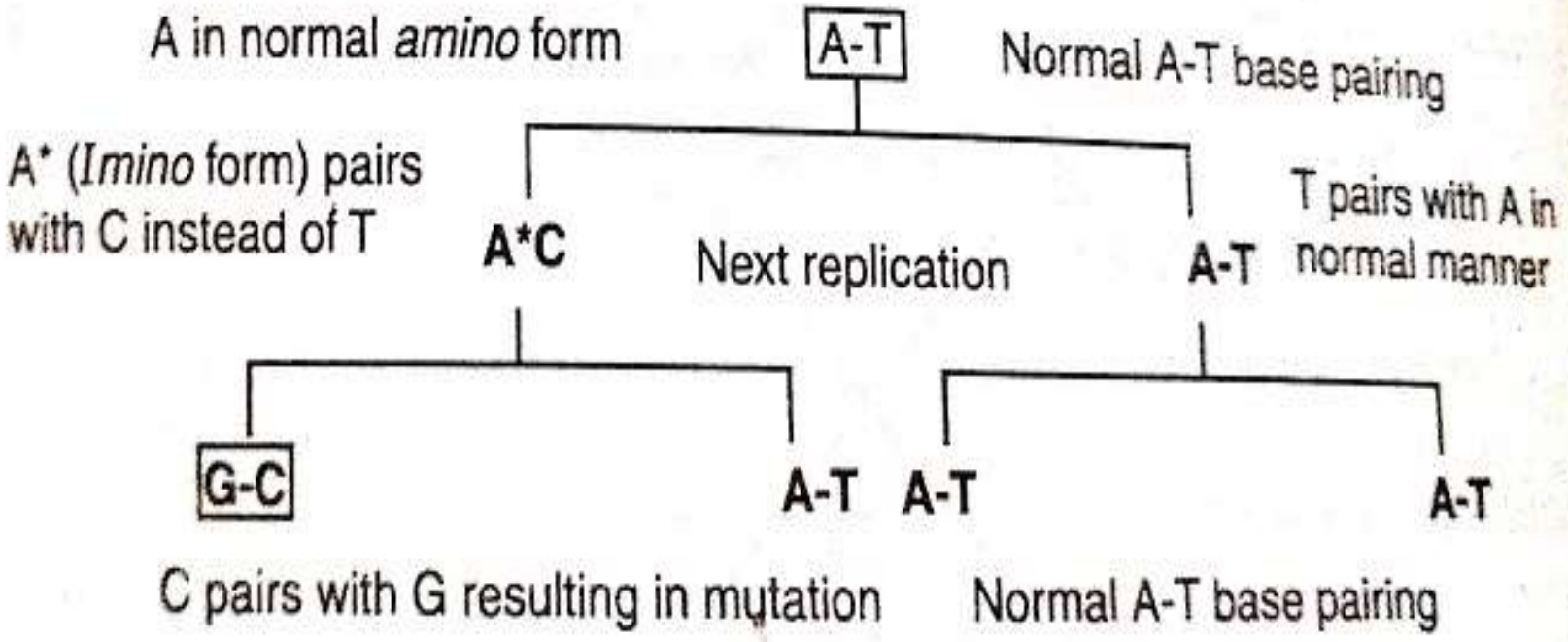


Fig. 18.5. Spontaneous mutation induced by tautomerism of A (normal *amino* form) to A* (*imino* form)

• One of the types of spontaneous gene mutations is forward mutation.
• Forward mutations result in changes away from the normal or wildtype.
• They include single base pair substitutions (transitions and transversions), mutations at protein level (neutral, missense and nonsense mutations), frameshift mutations (deletions and insertions), and multibase mutations (duplications, deletions/insertions, inversions and translocations)
• Base pair substitutions, mutations at protein level and frameshift mutations are examples of point mutations that involve one base.

❖ Transition: purine replaced by purine and pyrimidine replaced by pyrimidine.

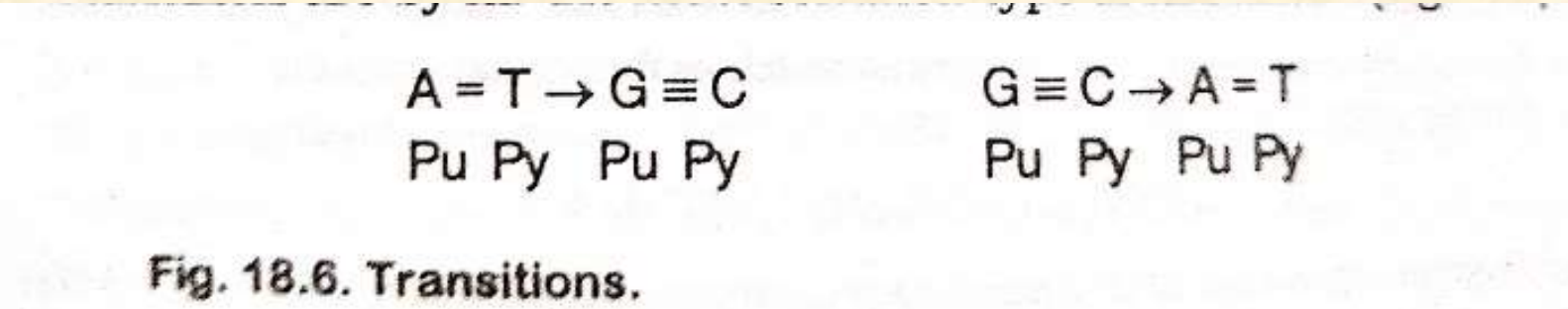


Fig. 18.6. Transitions.

❖ Transversion: purine replaced by pyrimidine or vice versa.

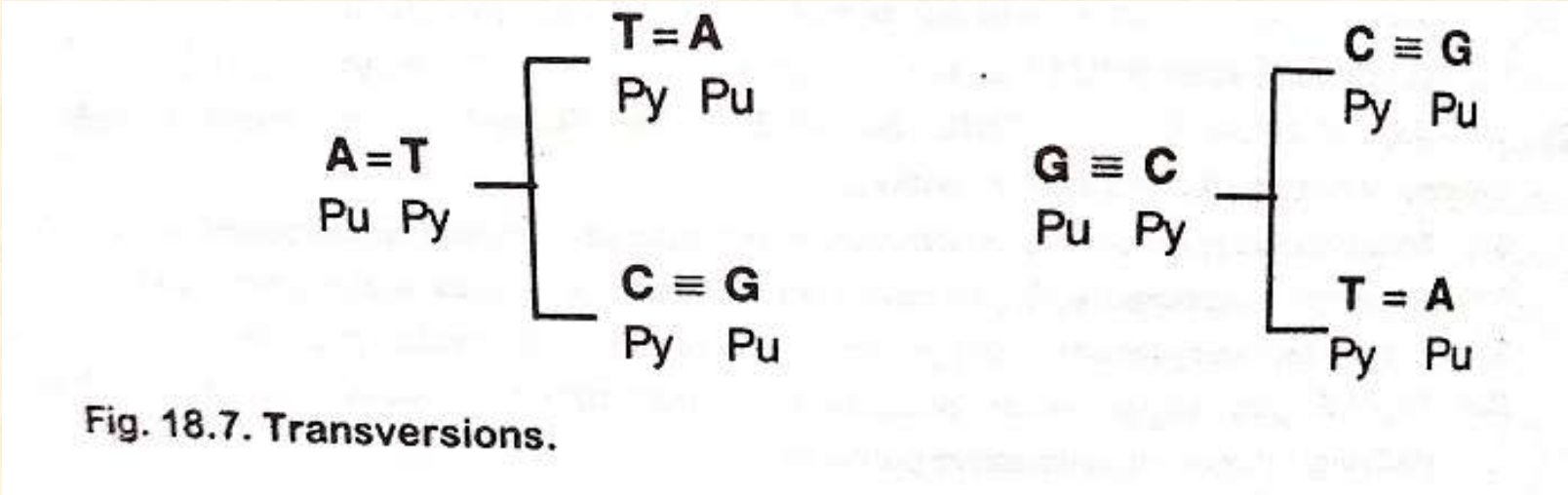


Fig. 18.7. Transversions.

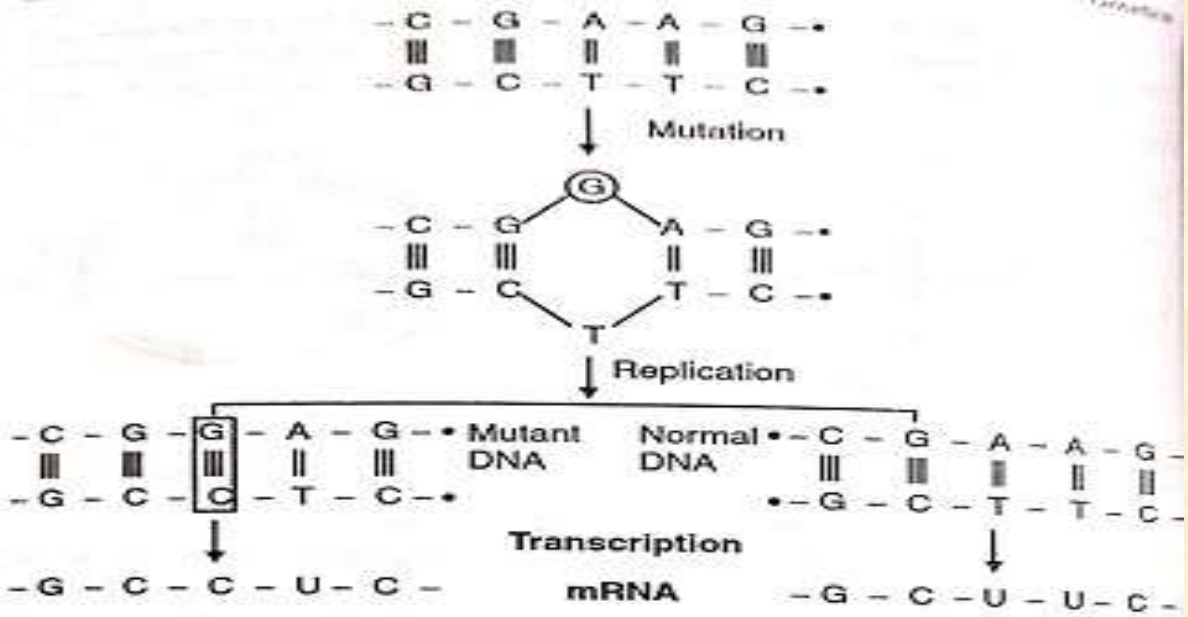


Fig. 18.9. Base substitution resulting from mutation.

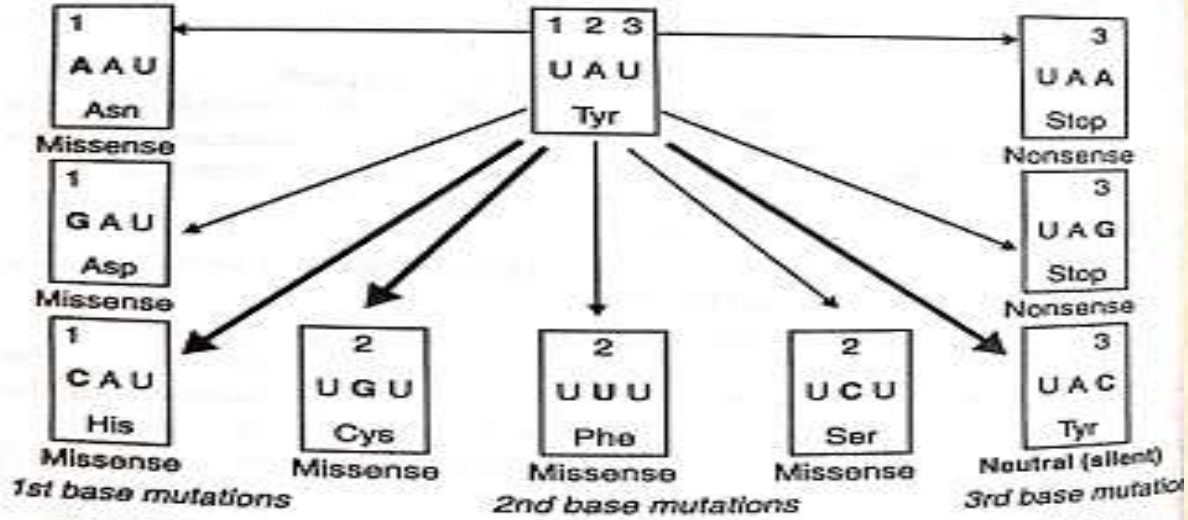


Fig. 18.10. Missense (6), nonsense (2) and neutral or silent (1) mutations resulting from a single base change in the tyrosine codon UAU. Light arrows indicate *transversions* and heavy arrows *transitions*.

• Multibase mutations involve several base pairs of DNA.

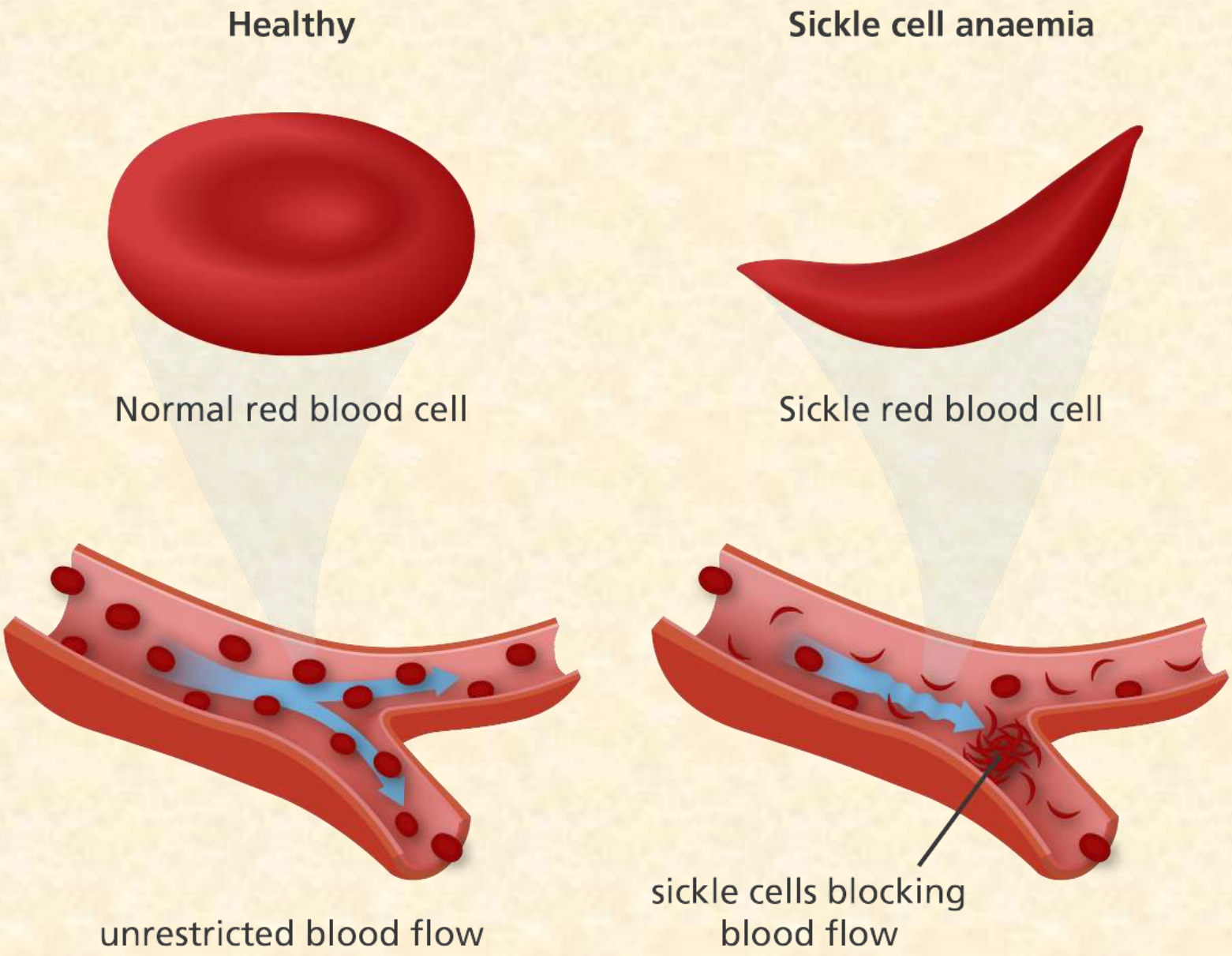
❖ Missense mutations:

• The sequence of amino acids in a polypeptide chain depends upon the base sequence of the mRNA from which it is translated.

• This in turn may lead to the insertion of a different amino acid in the polypeptide.

• If the alteration in the structure of the polypeptide leads to a detectable change in the phenotype, the result is called a missense mutation.

• Thus a missense mutation is one which results in the replacement of one amino acid in a polypeptide chain by another, with a resulting change in the phenotype.
• A missense mutation may be caused by substitution, deletion or insertion.
• Missense mutations arising by substitution may result in proteins which differ from their normal counterparts only in a single amino acid.
• The work of Ingram (1957) and of Hunt and Ingram (1958 ,1960) on the chemical differences between normal and variant haemoglobin has shown that only a single base pair is involved.


Healthy
Sickle cell anaemia
Normal red blood cell
Sickle red blood cell
unrestricted blood flow
sickle cells blocking
blood flow

• Human Hb is a protein molecule consisting of four polypeptide chains, two alpha and to beta chains.
• The alpha chain consists of 141 amino acid residues and the beta consists of 146 amino acid residues.
• The erythrocytes of normal persons are disc-shaped and contain hemoglobin A (Hb-A).
• Changes in hemoglobin structure result in a certain type of anaemia called sickle cell anaemia.
• The erythrocytes become sickle shaped when oxygen tension is reduced, and are much less effective in oxygen transport.

...ading in the molecular *stacking*.

Table 18.2. The first eight amino acids of the beta chain of four haemoglobin variants.

No.	Hb-A	Hb-S	Hb-C	Hb-G
1.	Valine	Valine	Valine	Valine
2.	Histidine	Histidine	Histidine	Histidine
3.	Leucine	Leucine	Leucine	Leucine
4.	Threonine	Threonine	Threonine	Threonine
5.	Proline	Proline	Proline	Proline
6.	Glutamic acid	**Valine**	**Lysine**	Glutamic acid
7.	Glutamic acid	Glutamic acid	Glutamic acid	**Glycine**
8.	Lysine	Lysine	Lysine	Lysine

Table 18.3. Hb-A : The first 8 DNA and mRNA codons and amino acid residues of the beta chain.

DNA codon	GTA CAT	CAT GTA	CTT GAA	ACT TGA	CCT GGA	GAA CTT	GAA CTT	AAA TTT
mRNA codon	GUA	CAU	CUU	ACU	CCU	GAA	GAA	AAA
Amino acid	val	his	leu	thr	pro	glu	glu	lys
Position	1	2	3	4	5	6	7	8

Table 18.4 . Possible mutations is variant haemoglobin DNA and consequent changes in amino acids.

	Hb-A	Hb-S	Hb-C	Hb-G
DNA codon	GAA CTT	GTA CAT	AAA TTT	GCA CCT
mRNA	GAA	GUA	AAA	GGA
Amino acid	Glutamic acid	Valine	Lysine	Glycine
Position	6 and 7	6	6	7

❖ Nonsense mutation:

• Of the 64 codons, 61 are sense codons i.e. they code for amino acids, while three are termination or nonsense codons which normally do not specify any amino acids.

• The three termination codons are UAA, UAG and UGA.

• Any mutation resulting in the alteration of a sense codon into a nonsense is called a nonsense mutation.

• Thus, if UAC the codon for tyrosine undergoes a one base substitution (C ⟶ G) it becomes UAG, a termination codon.

• A nonsense mutation brings about termination of the polypeptide chain at that point.

• As a result the polypeptide chain synthesized is incomplete.

• Such chains are biologically inactive.

• Such a nonsense mutation brings about a relatively drastic change in the protein synthesized, it is more likely to have a deleterious effect in the phenotype than a missense mutation.

❖ Frameshift mutation:

• A mutation in which there is deletion or insertion of one or few nucleotides other than 3 or multiples of 3 is called a frameshift mutation.
• The name is derived from the fact that there is a shift in the reading frame backward or forward by one or two nucleotide.
• Addition or deletion of one or two bases results in a new sequence of codons containing missense codons which may code for entirely different amino acids.

• This results in a drastic change in the protein synthesized. The protein is usually nonfunctional.
• The frameshift may also result in nonsense codons resulting in a chain termination.

▪ Deletion:

Original message or reading frame
CAT GAT CAT GAT CAT GAT CAT
Deletion - C
CAT GAT **ATG ATC ATG ATC AT**

- Insertion :

Insertion +G

CAT GAT **GCA TGA TCA TGA TCA T**

- Simultaneous deletion and addition :

Deletion and insertion

-C +C

CAT GAT **ATG ATC ATC** GAT CAT

Original sequence:

CAT GAT CAT GAT CAT GAT CAT