Molecular Clock

1) Define Molecular Clock

Mutational changes of biomolecule over divergence time is called molecular clock.

Or, Mutation rate of biomolecule over divergence time is called molecular clock.

2) Explain the molecular clock

i) The molecular clock is established on the hypothesis of "The rate of molecular evolution is equal to the rate of neutral mutation (Kimura, 1968)" and the rate of molecular evolution is constant over divergence time (Kimura, 1968)."

ii) Molecular evolution includes changes of nucleotide sequence in DNA and amino acid sequence in protein and the molecular data are nucleotide sequences for DNA and amino acid sequences for proteins.

iii) This change is the function of nucleotide substitution or amino acid substitution under the governance of mutation force.

iv) The number of mutation of DNA or protein molecules are quantified as number of nucleotide substation in a gene or number of amino acid substitution in a protein over divergence time (millions of year).

v) If number of mutations are plotted against divergence time of the molecule, a graph is prepared that is molecular clock. It shows the rate of mutation of the molecule. So, each molecule has a specific graph.

m	μ - mutation rate
$\mu = \frac{m}{2t}$	m – number of mutation
21	t – divergence time

vi) All these graphs are linear. It states that the mutation rate of a gene or protein is constant over divergence time. Accordingly, the rate of molecular evolution is constant among lineages.

$\mu = \frac{m}{2t} = c$	c – coi	nstant
or, μ=c	where,	the value of c is molecule specific or species specific





Molecular Clocks



vii) It can also predict the rate of molecular evolution that equals with neutral mutation rate.

k = 2N μ x and, x = $\frac{1}{2N}$ Then, k = 2N . μ . $\frac{1}{2N}$ = μ So, k = μ k - rate of evolution
μ - neutral mutation rate
N - population size of diploid organism

x - probability of fixation of a neutral mutant allele

viii) It is calculated that the genetic difference between two species is proportional to their divergence time from their common ancestor. It is also true for protein molecules.

 $\mu = \frac{d}{2t} \qquad \qquad \mu \text{-mutation rate} \\ d - \text{number of genetic or amino acid difference} \\ t - \text{divergence time}$





Figure 7.8 DNA mutates at a roughly clock-like rate. This graph shows how distantly related pairs of species have a large number of different substitutions in the cytochrome c gene. (Adapted from Moore and Moore, 2004)



ix) The expected time for speciation is calculated by the following formula -

$$t = \frac{m}{2\mu}$$
 or, $t = \frac{d}{2\mu}$

t-divergence time $m-number\ of\ mutation$ $d-number\ of\ genetic\ or\ amino\ acid\ difference$ μ - Neutral mutation rate

x) If fossil records are available then divergence time is calibrated.





Who did coin the term "Molecular Clock"?
 Zuckerkandl and Pauling coined "Molecular Clock" in 1965.

- 4) Give significance of molecular clock
- i) It can calculate the time of speciation or radiation.
- ii) Molecular clock is an important tool in molecular systematics.
- iii) It facilitates in formation of phylogenetic tree

5) Cite some limiting factors of molecular clock.

i) Changing generation time : The rate of genetic mutation is generation - time dependant. Species with shorter generation time evolves higher mutation rate. Whereas, mutation rate of protein molecule is independent to generation time.

ii) Population size (small size) small population has higher rate of molecular substitution due to genetic drift and ineffectiveness of selection force.

iii) More species specific differences like metabolism, ecology, evolutionary history etc. encourage higher number of mutation.

- iv) Functional efficiency of protein is proportional to neutral mutation rate.
- v) Intensity of natural selection is proportional to neutral mutation.

6) Mention the drawback of molecular clock.

Choice of calibration has a substantial influence on the outcome of molecular clock analysis. Calibration methodology can lead to estimate erroneous time scale.

7) Briefly describe the α -globin molecular clock

Both α -globin gene and α -globin protein in vertebrate can be used to construct a molecular clock. All vertebrates have two identical α - globin genes – α_1 and α_2 . These genes were originated from jawless fish (common ancestors) approximately 450 – 500 million years ago. Number of amino acid in α - globin in shark is 62 and in human 141. This number increases gradually over

divergence time. Plotting of the number of amino acid difference against divergence time makes a molecular clock that predicts the rate of neutral mutation and the rate of evolution.

 $\mu = \frac{d}{2t} \qquad aa_1 - 62$ Then, $\mu = \frac{141 - 62}{2 \times 440} \qquad aa_2 - 141$ Then, $\mu = \frac{79}{880}$ Then, $\mu = 0.09$

So, mutation rate of α -globin is 0.09 amino acid/million year

As, $k = \mu$

Then, k = 0.09

So, the rate of evolution of α -globin is 0.09 amino acid/million year

A phylogenetic tree can be formed based on number of amino acid difference in α -globin protein among different vertebrate species.

Speciation is expected at least one amino acid difference i.e., d = 1

So, t = $\frac{d}{2\mu}$ Then, t = $\frac{1}{2 \times 0.09}$

Then, t = 5.56

So, new species may be originated after 5.56 million years after human origin.



Each point represents the number of substitutions separating each animal from humans





Evolutionary history of the globin genes



Table: Number of amino acid difference in haemoglobin protein among mammals

	α man	α horse	α cattle	βman	β horse	β cattle	γ man	γ cattle
α man	0	17	27	74	81	75	79	82
α horse	17	0	38	77	85	77	77	77
α cattle	27	38	0	81	83	83	81	88
βman	74	77	81	0	26	27	39	32
β horse	81	75	83	26	0	35	43	33
β cattle	75	77	83	27	35	0	45	25
γ man	79	77	81	39	43	45	0	40
γ cattle	82	77	88	32	33	28	40	0

Cytochrome c molecular clock for phylogeny

		Evolution of Cytochrome c	
Species c	omparison	Number of variant residues	Divergence of lines in millions of years
Horse	— Man	12	130
Horse	- Pig	3	33
Horse	- Chicken	12	108-150
Pig	- Chicken	10	
Rabbit	- Chicken	11	
Man	- Chicken	14	
Horse	— Tuna	19	101.000
Pig	— Tuna	17	
Rabbit	- Tuna	19	184-228
Man	- Tuna	21	
Chicken	- Tuna	18	
Horse	— Yeast	44	
Pig	— Yeast	43	
Rabbit	— Yeast	45	465-520
Man	— Yeast	43	
Chicken	- Yeast	43	
Tuna	- Yeast	48	

(b) The graph shows a molecular clock which compares the amino acid sequence of the protein cytochrome c between a range of species.



(i) Cytochrome c is a protein containing 112 amino acids.

Calculate the number of amino acids in cytochrome c that are different between two species whose last common ancestor lived 500 million years ago.

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rRNA molecular clock for phylogeny

