B.Sc (Hons) Microbiology (CBCS Structure)

C-7: Molecular Biology

Unit 1: Structure of DNA and RNA/ Genetic Material

Denaturation/Renaturation Cot An

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Reference – Molecular Biology of the Gene (6th Edition) by Watson et. al. Pearson education, Inc
Principles of Genetics (8th Edition) by D. Peter Snustad, D. Snustad, Eldon Gardner, and Michael J. Simmons, Wiley publications

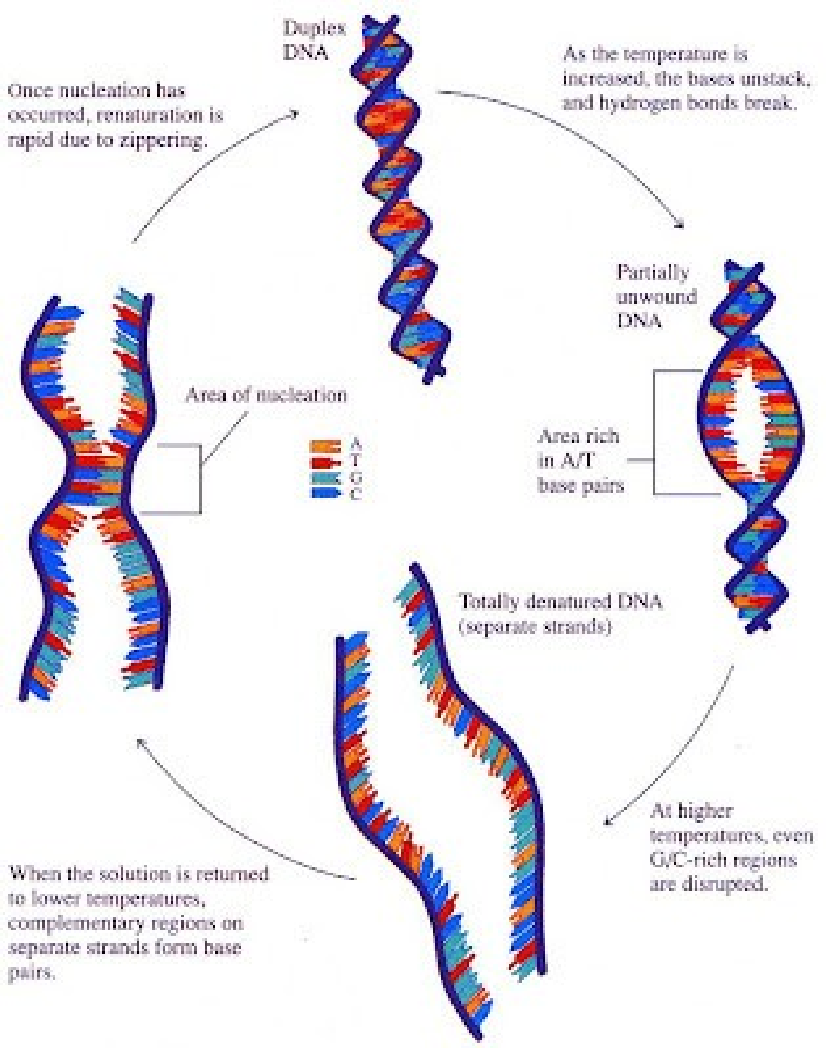
DNA strands can separate and re-associate

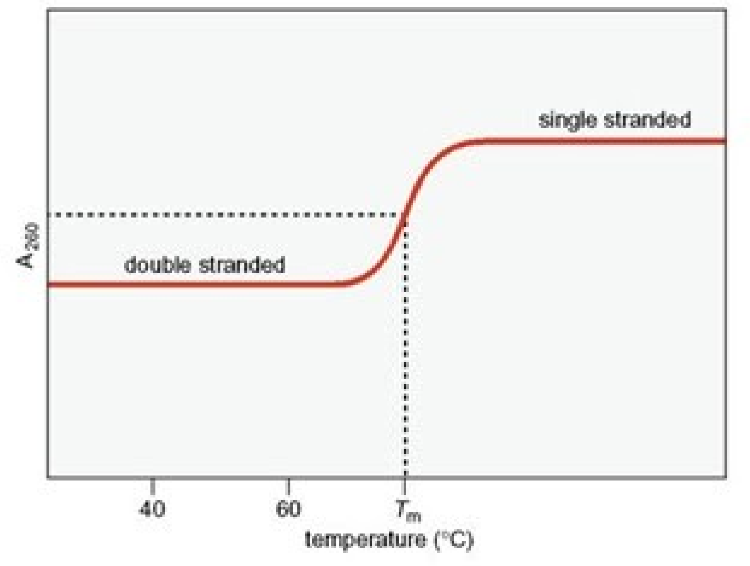
Key terms to understand

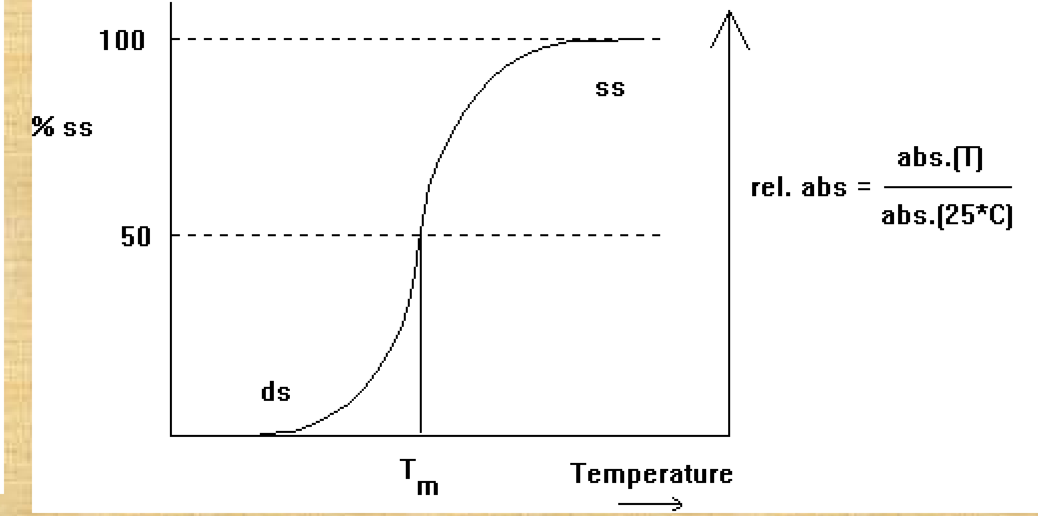
1. Denaturation
2. Hybridization
3. Annealing/renature
4. Absorbance
5. Hyperchromicity
6. Tm (melting point)

High GC content and high salt concentrations of solution increases the melting temperature
GC content – Three H bonds and stacking interactions with neighboring base pairs

High salt concn. Shielding the –vely charged phosphates on two strands







At a wavelength of 260 nm, the average extinction coefficient for double-stranded DNA-0.020 (μg/ml)$^{-1}$ cm^{-1}
single-stranded DNA - 0.027 (μg/ml)$^{-1}$ cm^{-1},single-stranded RNA it is 0.025 (μg/ml)$^{-1}$ cm^{-1}
50 μg/ml for double-stranded DNA – OD 1.0, 50 μg/ml for single-stranded DNA – OD 1.35

Denaturation and Renaturation of DNA

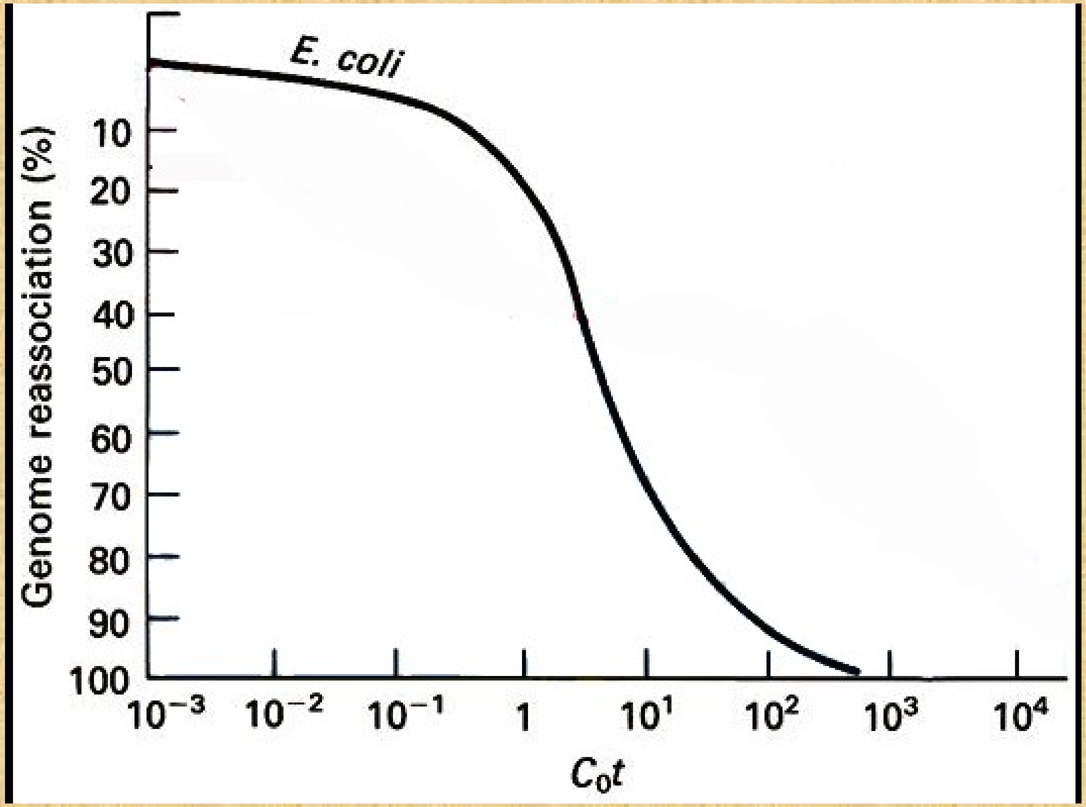
The rate at which the DNA reanneals (another term for renature) is a function of the species from which the DNA was isolated

The Y-axis is the percent of the DNA that remains single stranded. This is expressed as a ratio of the concentration of single-stranded DNA (*C*) to the total concentration of the starting DNA (*Co*).

The X-axis is a log-scale of the product of the initial concentration of DNA (in moles/liter) multiplied by length of time the reaction proceeded (in seconds).
The designation for this value is *Cot* and is called the "Cot" value. The curve itself is called a "Cot" curve.
The curve is rather smooth which indicates that reannealing occurs gradually over a period of time.
One particular value that is useful is *Cot½* , the *Cot* value where half of the DNA has reannealed.

Cot Curves

Co= 10^{-3} moles/L=1mM
t = sec.



- a sigmoid curve which can be characterized by the **Cot1/2** value, the point where 1/2 of the DNA is still single stranded.

Cot Analysis

- **In this reaction the rate-limiting step is the collision of two complementary molecules, giving second-order reaction kinetics.**

- **The rate of collisions is proportional to initial concentration (Co) times time (t), or Cot.**

- **a collision will result in formation of a Double Stranded molecule depending on whether the two strands are complementary.**

Steps Involved in DNA Denaturation and Renaturation Experiments

1.Shear the DNA to a size of about 400 bp.
2. Denature the DNA by heating to 100°C.
3. Slowly cool and take samples atdifferent time intervals.
4. Determine the % single-stranded DNA at each time point.

The shape of a "Cot" curve for a given species is a function of two factors:
the size or complexity of the genome; and
the amount of repetitive DNA within the genome

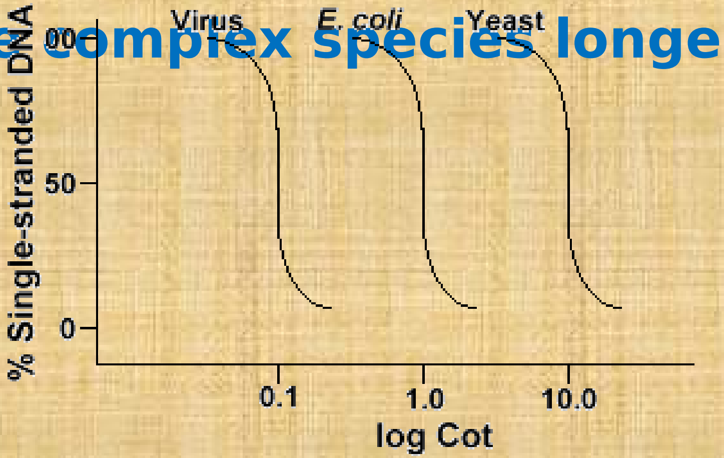
If we plot the "Cot" curves of the genome of three species such as bacteriophage lambda, *E. coli* and yeast we will see that they have the same shape, but the *Cot½* of the yeast will be largest, *E. coli* next and lambda smallest.

Physically, the larger the genome size the longer it will take for any one sequence to encounter its complementary sequence in the solution.

This is because two complementary sequences must encounter each other before they can pair.

The more complex the genome, that is the more unique sequences that are available, the longer it will take for any two complementary sequences to encounter each other and pair.

Given similar concentrations in solution, it will then take a more complex species longer to reach Cot½ .



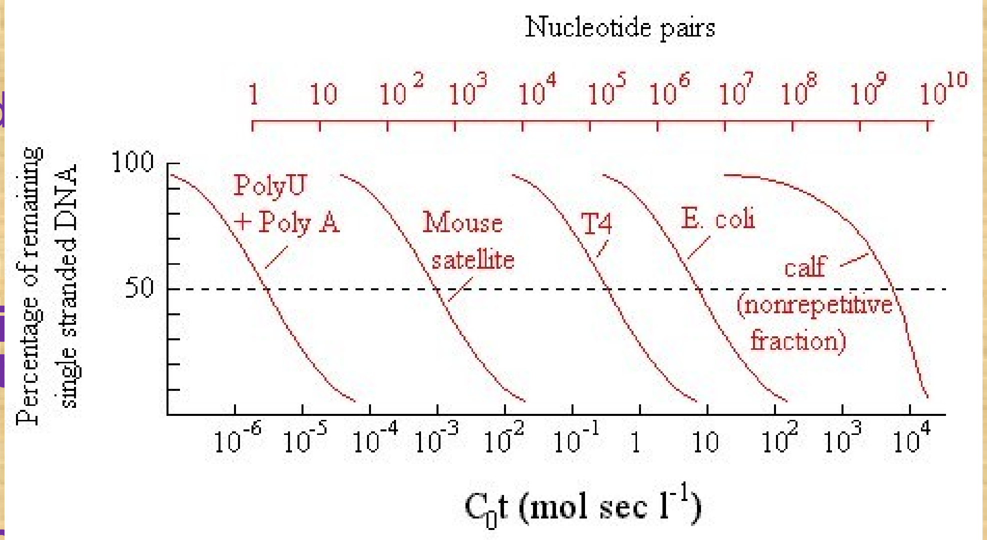
Cot Curves and Copy Number

- **Number of copies of each sequence determines the rate: i.e how many collisions does a given strand have to make before it finds a match.**

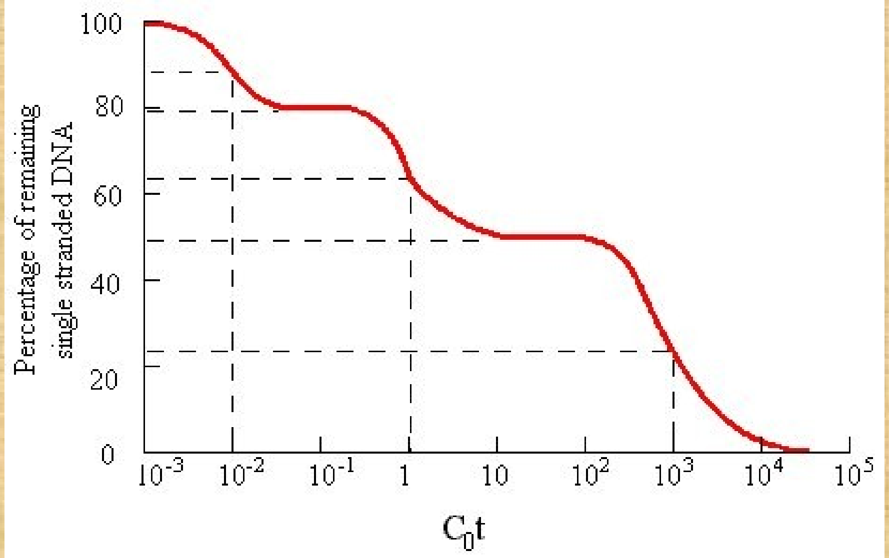
- **For example, if one stand is all U's (poly U) and the other strand is all A's (poly A), on the average only 2 collisions will occur before a strand find a match.**

- **For 50 kb phage DNA cut into 1 kb lengths, only 1 collision i 100 will result in a match: Cot ½ is bigger.**

- **For 4 Mbp E. coli genome, one collision in 8000 will be productive.**



Complex Cot Curves



- For eukaryotic DNA, Cot curves are not simple sigmoid curves.
- Computer analysis generally resolves them into 3 characteristic portions of the genome with each component having its own Cot1/2 value
    - highly repeated DNA: average of 50,000 copies per genome, about 10% of total DNA
    - moderately repeat DNA: average of 500 copies, a total of 30% of the genome
    - unique sequence DNA: up to 10 copies: about 60% of the genome.

What Repeat Classes Represent

- **Unique DNA:**
  - **highly conserved coding regions: 1.5%**
  - **other highly conserved regions: 3%**
  - **other non-conserved unique sequences: 44%**

- **Moderately repeated DNA**
  - **transposon-based repeats: 45%**
  - **large gene families**

- **Highly repeated DNA:**
  - **constitutive heterochromatin: 6.6%**
  - **microsatellites: 2%**
  - **a few highly repeated transposon families (Alu sequences)**

Species	Sequence Distribution
Bacteria	99.7% Single Copy
Mouse	60% Single Copy 25% Middle Repetitive 10% Highly Repetitive
Human	70% Single Copy 13% Middle Repetitive 8% Highly Repetitive
Cotton	61% Single Copy 27% Middle Repetitive 8% Highly Repetitive
Corn	30% Single Copy 40% Middle Repetitive 20% Highly Repetitive
Wheat	10% Single Copy 83% Middle Repetitive 4% Highly Repetitive
Arabidopsis	55% Single Copy 27% Middle Repetitive 10% Highly Repetitive