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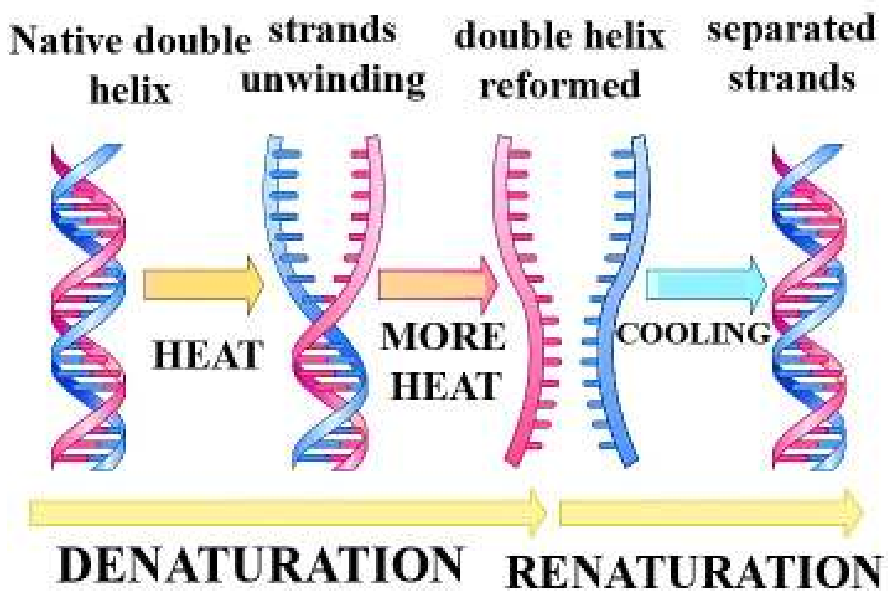
DNA Denaturation and Renaturation

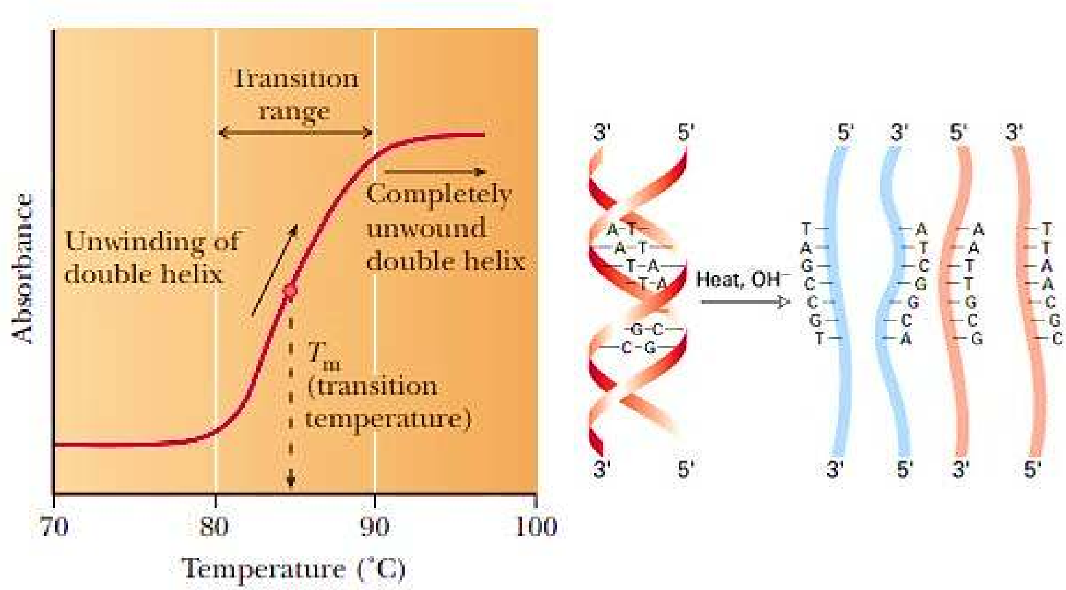


DNA Denaturation

- The unwinding and separation of DNA strands, referred to as **denaturation, or "melting,"** can be induced experimentally by **increasing the temperature of a solution of DNA or by changing the pH.**

- As the **thermal energy increases**, the resulting increase in molecular motion eventually **breaks the hydrogen bonds and other forces that stabilize the double helix;** the strands then separate, driven apart by the electrostatic repulsion of the negatively charged deoxyribose-phosphate backbone of each strand.

- **Near the denaturation temperature**, a small increase in temperature causes a rapid, near **simultaneous loss of the multiple weak interactions holding the strands together along the entire length of the DNA molecules.**



The temperature at which the DNA strands are ½ denatured is the melting temperature or T_m

GC content of DNA has a significant effect on T_m with higher GC content yielding a higher T_m

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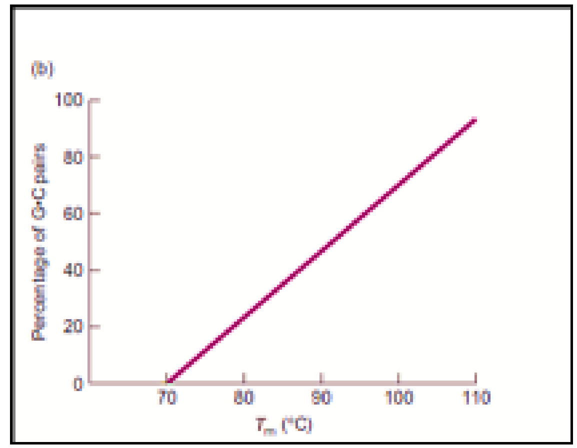
The melting temperature T_m at which DNA strands will separate depends on several factors

- Molecules that contain a **greater proportion of G·C pairs require higher temperatures to denature** because the three hydrogen bonds in G·C pairs make these base pairs more stable than A·T pairs, which have only two hydrogen bonds.
- The **ion concentration** also influences the T_m because the negatively charged phosphate groups in the two strands are shielded by positively charged ions. **When the ion concentration is low, this shielding is decreased, thus increasing the repulsive forces between the strands and reducing the T_m.**
- Agents that destabilize hydrogen bonds, such as **formamide or urea, also lower the T_m.**
- Finally, **extremes of pH denature DNA at low temperature**. At **low (acid) pH**, the **bases become protonated and thus positively charged, repelling each other.**
- At **high (alkaline) pH,** the bases **lose protons and become negatively charged, again repelling each other because of the similar charge.**



DNA Renaturation

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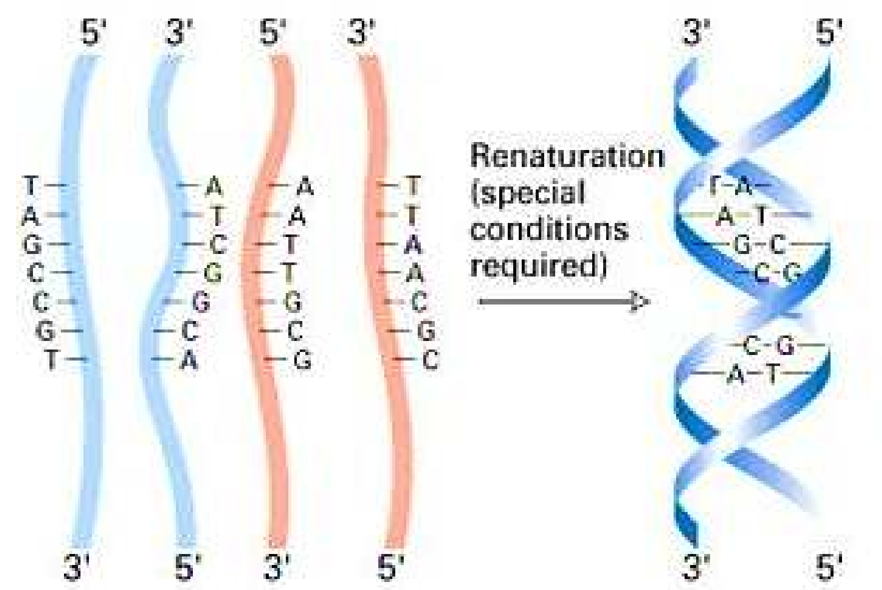
- The single-stranded DNA molecules that **result from denaturation form random coils without an organized structure.**
- **Lowering the temperature, increasing the ion concentration, or neutralizing the pH** causes the two complementary strands **to reassociate into a perfect double helix.**
- The extent of such renaturation is **dependent on time, the DNA concentration, and the ionic concentration.**
- Two **DNA strands not related in sequence will remain as random coils and will not renature;** most importantly, they will not inhibit complementary DNA partner strands from finding each other and renaturing.



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DENATURATION OF DNA VERSUS RENATURATION OF DNA

DENATURATION OF DNA	RENATURATION OF DNA
The unwinding of the double-stranded DNA by the breaking down of hydrogen bonds, which hold the two DNA strands together	The formation of base pairs, coming back two complementary strands of DNA together
Gives rise to single-stranded DNA	Gives rise to double-stranded DNA
Breaks down hydrogen bonds between complementary base pairs	Forms hydrogen bonds between complementary base pairs
Factors: Chemical agents such as formamide, DMSO, and alkaline and physical agents such as heating and sonication	Factors: Ionic strength of the solution, temperature, time, DNA concentration, and the size of the interacting molecule
Increases the absorbance of DNA at 260 nm	Decreases the absorbance of DNA at 260 nm
Highly decreases the positive optical rotation of the DNA double helix	Gives a strong, positive optical rotation to the DNA double helix
Remarkably decreases the viscosity of DNA	Highly increases the viscosity of DNA

Hyper and Hypochromic shift

- The **close interactions between stacked bases in a nucleic acid** has the effect on **decreasing its absorption of UV light** relative to that of a solution with the same concentration of free nucleotides and the absorption is decreased further when two complementary strands are paired. This is called **hypochromic shift.**
- **Denaturation of double stranded strand** produces the **opposite result and increase in absorption of UV** and called **Hyperchromic shift.**