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'Hyperchromicity and Strand Separation in Bacterial DNA'- A Literature Review

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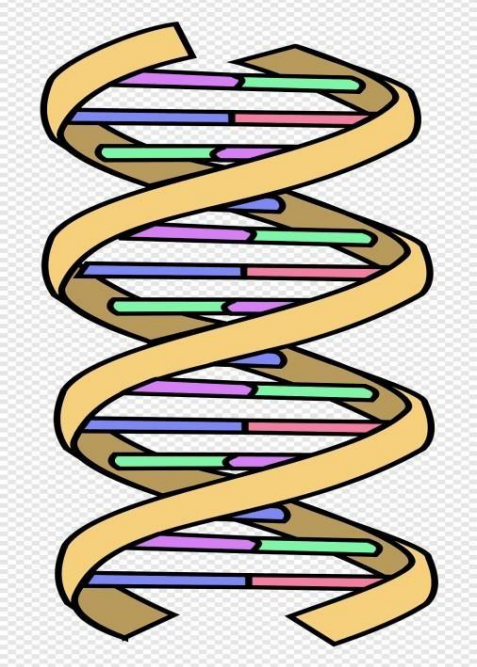
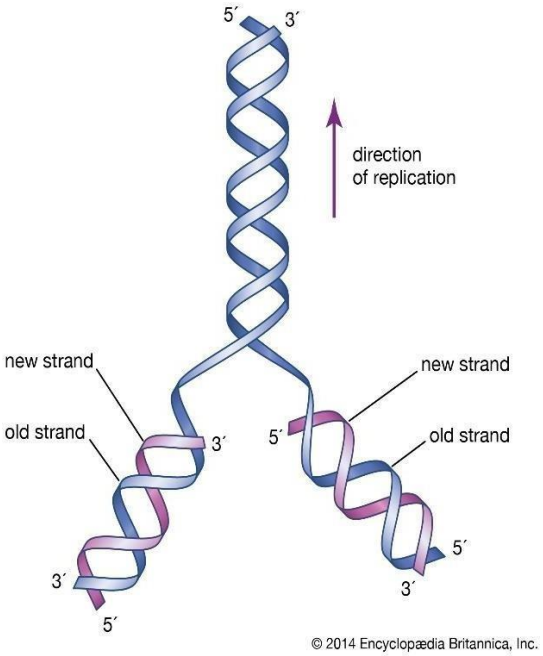
I. Introduction:



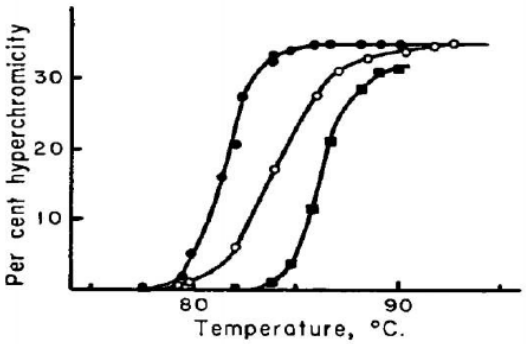


If a solution containing duplex DNA molecule is either heated or treated with alkali, thehydrogen bonds between the two strands become unstable and the two strands start to separate this is known as denaturation or melting. Hyperchromicity is the increase of absorbance/ optical density of a material. The UV absorption is increased when the twosingle DNA strands are being separated, either by heat or by addition of denaturant or by lowering the pH level. The strand separation being due to increase inhyperchromicity has been related to the breakdown of hydrogen bonds between complementary base pairs. A graph drawn representing the relationship between optical density and temperature is called a "melting-out curve". The breadth of the curve is taken into consideration and it is been analysed through experimental study that the temperature range between the initiation and the completion of denaturation is less in case of the synthetic adenine-thymine polymer than for any other DNA preparation studied. The breadth of the melting-out curve changes due to the variations in the base distributions in the DNA molecules.

Two broad situations in melting-out of DNA have been observed but it isn't clear which of the two contributes more to the breadth of the curve. The first one being- the separation of the strands of the double helix at any one point in the molecule leads immediately to the complete denaturation of that molecule, and the other being- each short sequence of bases along a molecule melts out at a temperature determined by the local base composition. It is little evident that the latter situation contributes more to thebreadth of the curve.

For instance, T2 and T7 bacteriophage DNA, which are physically homogeneous melt out across a temperature range which is not much narrower than that of bacterial and animal DNA preparations. Each DNA has a characteristic mean density. Thus, it can beinterpreted that there are no molecules with densities which occupy the extremes of themelting range. The denaturation process is studied and analysed by detecting the presence of N^{14} and N^{15} single strands from a hybrid N^{14}-N^{15} DNA preparation under such experimental conditions where

none of the base pair hydrogen bonds reform on cooling.



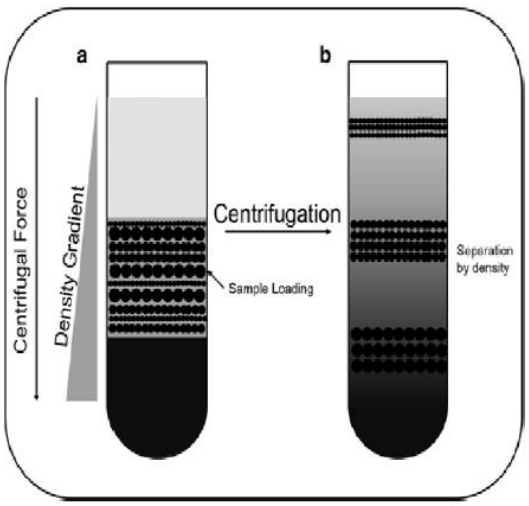
Melting-out curves of trout sperm, T2, and T7 bacteriophage DNA preparations in 0.15 M sodium chloride (NaCl), 0.015 M sodium citrate.

- ○ Trout sperm
- ▪ T7 bacteriophage

Aim of the study: To understand how the process of strand separation is related to themeasured hyperchromicity.
Methods: The experiment was performed using 3 experimental techniques:

1. **Growth of N^{14}-N^{15} Bacteria and Isolation of the Hybrid DNA**: Meselson's and Stahl's method was modified to grow E. coli B which was containing N^{14}-N^{15} hybrid DNA. Bacteria that were cultured in N^{15} synthetic medium was diluted witha rich N^{14} medium. When the titer had doubled (10^9/ml), growth was stopped with iced 0.01 M sodium azide. The bacteria were concentrated to 5 x 10^{10}/ml in 0.01 Msodium benzoate, 0.01 M versene, pH was maintained at 7, and heated for 20 minutes at 70°C to inactivate enzymes. This method was employed to reduce singlestrand breaks. DNA was isolated by the phenol technique and digested with boiled ribonuclease and repeated ethanol precipitation.
2. **Denaturation**: The denaturing solution was 0.01 M phosphate, pH 7.8, containing 1, 2, or 4 per cent freshly neutralized (pH 7 - 8.5) HCHO. 0.02 ml DNA (80 ug/ml)was added to 0.2 ml denaturing solution and heated immediately in 2 ml sealed ampoules for 10 minutes, keeping DNA in formaldehyde at neutral pH prevents theseparation of strands. Optical density is time dependent for a specific temperature, this fact was a priority here. Optical density was measured before and after denaturation against appropriate HCHO solutions.
3. **CsCl Density Gradient Centrifugation**: The solution composed of 0.787 gm recrystallized CsCl and 0.584 gm denaturing solution containing 2 to 4g bacterial DNA and 2 jug phage T2 DNA which was being used as a density marker in the study. All runs were at 44,770 RPM and 250C in a Spinco Model E ultracentrifuge with ultraviolet absorption optics.

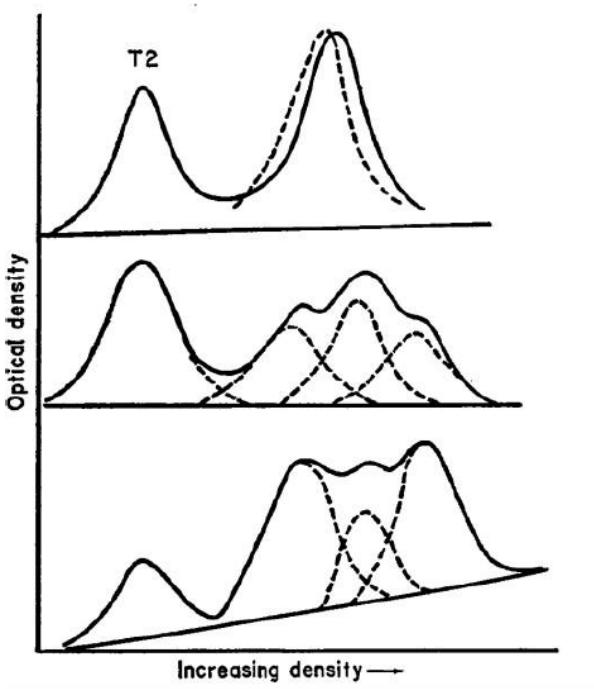
The photographs were scanned with a Joyce-Loebl recording microdensitometer. Average concentrations of single and double stranded DNA was noted by studying the areas under the peaks.



Joyce-Loebl recording microdensitometer

II. Results:

The degree of hyperchromicity that was achieved did not reduce on cooling which means the hydrogen bonds do not reform on cooling. No aggregation of strandsoccurs after heating in formaldehyde.

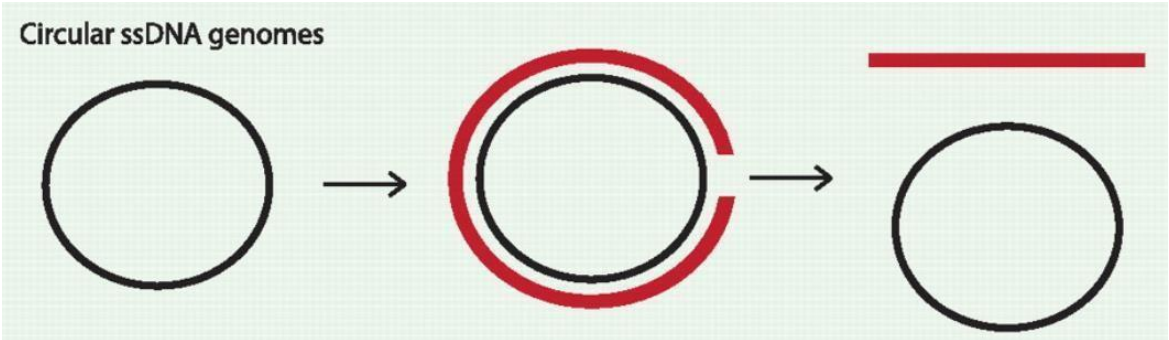


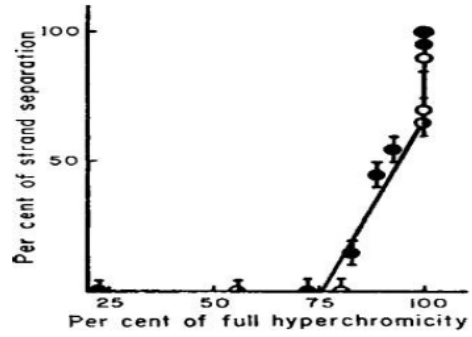
Densitometer traces of banded hybrid coli DNA at 30, 87, and 100 per cent of full hyperchromicity. The dashed curve in the first diagram indicates the banding position of the undenatured DNA. The dashed curves in the lower diagrams indicate the approximate areas assigned to the N^{14}, hybrid, and N^{15} bands. T2 DNA is the marker in each diagram.

As denaturation proceeds, the density of the partially dissociated hybrid DNA increased. The density depends on the concentration of formaldehyde in the CsCl solutions. It can be understood from below data:

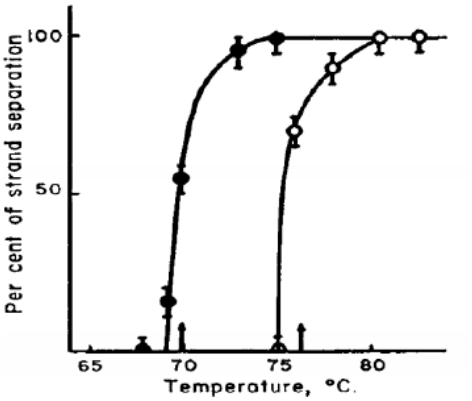
Formaldehyde concentration inbanding solution [% cent]	**Increase in density on denaturation[gm/ml]** 0.015
0	
1	0.012 ± **0.0005**
4	0.0045 ± **0.0005**

Similar results were obtained when experiments were performed on two other hybrid DNA preparations which contained small amounts of heavy or light DNA, but in this itwas more difficult to analyse the stand separation.





Percentage of the strand separation as a function of hyperchromicity in hybrid DNA heated for 10 minutes in solutions of 1 per cent (O), 2 per cent (half-filled circle) and 4 per cent (filled circle) formaldehyde. The uppermost point (100 per cent strand separation) represents the superposition of results from many experiments in 1, 2, and 4 per cent



Relationship between the percentage of strand separation in hybrid DNA and the incubation temperature for samples heated in 1 per cent (O) or 4 per cent (filled circle) formaldehyde for 10 minutes. The arrows indicate the onset of full hyperchromicity.

As the two curves are almost similar in shape and orientation, it can be assumed on the basis of this experimental study that strand separation is related to hyperchromicity and not just temperature or the formaldehyde concentration. Strand separation is not observed below 75% of hyperchromicity and also for complete separation, 100% hyperchromicity is not a must criterion. Although it is a fact that formaldehyde can induce cross-link formation between strands, but here it does not seem to cause such links, they do not melt at 100°C too. It is assumed that there exists short guanine- cytosine rich, hydrogen bonded sequences which are very negligible for hyperchromicity and melt out at temperature little above the melting-out curve.

III. Conclusion:

These studies have shown that strand separation is dependent on temperature and formaldehyde concentration which are directly related to the measuredhyperchromicity. For separation to happen, at least 75% of full hyperchromicity is needed. Even at maximum hyperchromicity, little amount of the strands remains together probably due to the guanine-cytosine rich regions. As single strand breaks were present along the molecules during hydrolytic degradation while heating, it indicates clearly that single N^{14} or N^{15} strands would be detected before the complete unwinding of the molecule occurs. The percentage of complete hyperchromicity in presence or absence of formaldehyde does not always indicate the extent to which the hydrogen bonds break.

Meselson and Stahl have proved that bacterial DNA replicates semi-conservatively andthe replicated subunits can be separated from each other by heating to 100°C in 7.7 M CsCl. They also assumed that heating to 100 °C in CsCl breaks the 'biunial' bonds which hold the two double stranded molecules together and these bonds can be broken only when they are heated in CsCl, but their experiments have revealed that heating at 100°C in the absence of CsCl also results in the appearance of the N^{14} and N^{15} bands.

Similar experiments performed by Marmur and Ts'o at room temperature and by Schildkraut, et.al in acid and alkali at room temperature have yielded desired results andit was thus stated that the separation of the subunits requires neither CsCl nor 100 °C temperature. Their experiments indicate that the bonds break depending on the conditions relating to the degree of hyperchromicity. The criteria of full hyperchromicity is not a must criteria in every case. For example, heating to 100 °C inionic strength which is more than 0.2 is not sufficient always for complete separation of subunits in DNA with a high guanine-cytosine content to occur.

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Bibliography:

[1]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1366370/pdf/biophysj00656-0015.pdf
[2]. https://pubs.acs.org/doi/10.1021/acs.jchemed.6b00095
[3]. https://pubmed.ncbi.nlm.nih.gov/23799235/

David Freifelder, et. al. "Hyperchromicity and strand separation in bacterial DNA." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, 8(6), (2022): pp. 31-35.