S U M M A R Y

The content of the thesis has been divided into four chapters.

1) The first chapter traces the historical development of reassociation of DNA to its present day position as a principal tool in molecular biology. The discovery of repeated DNA sequence, its universality as a bulk content of the eukaryotic genome and its sequence organisation have been discussed. The uniform patterns of interspersion of repeated and unique sequences among various organisms have been impressed upon. The concept of "selfish DNA" discarding the middle repetitive sequence a role to play in cellular process has been modestly criticised.

The variety of information which one can get from the kinetics of DNA reassociation has been briefly mentioned.

This chapter ends with a discussion on reassociation kinetics, the technique proper. It has two faces. Firstly, the conditions of reassociation and the methodology of building the kinetic equation are discussed. Secondly the effect of various environmental parameters is analysed. The effect of such variables as temperature, fragment length, ionic environment and others are critically assessed.

2) The second chapter deals with the building up of various kinetic equations. Generally the description of the

kinetic equation for reassociation is dependent on the tool that monitors reassociation. The various kinetic equations that have been discussed and derived in this chapter follow the reaction as monitored by HAP chromatography.

The equation describing inter species DNA-DNA hybridization is first derived. This derivation is based on the frame work of the equation describing DNA-reassociation as proposed by Britten et al. Solutions of these equations (reassociation and hybridization) are also proposed.

The effect of real parameters on reassociation are also analysed. These are random breakages and length distribution of DNA fragments. Due to the dependence of rate constant on fragment length (complementary overlap between two strands to be precise) the kinetic equation is modified under the influence of these real causes. Taking a real world length distribution and concerting with it the effect due to random breakage-the modified rate equation is described. The numerical solution of this equation is discussed along with its various implications. Two important points arise for the solution - firstly it shows that even under the real world perturbations of random breakage and length distribution the reaction seems to follow second order kinetics. Secondly the rate constant is described not by the average length of the DNA fragments (as is generally thought) but by a value which is significantly lower. The quasi-analytical solution

of this equation under some major restrictions is also worked out.

Due to the fact that reassociated duplex contains single strand tails, tail-single strand interaction is an observed phenomenon. This should deviate the ideal reaction from its second order kinetics. The modified equation taking into account tail interaction is proposed. The analytical solution of this equation shows that during the major part (about 60%) of the reaction the kinetics are indistinguishable from the ideal reaction. Measurable difference occurs only in the later stage of the reaction.

3) The third chapter mainly deals with various experiments and the discussion of the results. Optical melting of reassociated DNA, and presenting the data in a derivative plot gives interesting information about the precise matching of sequences in reassociated duplex. The melting of reassociated calf thymus DNA is non-cooperative and melting temperature (T_m) is lowered by about 8°C, whereas the melting of reassociated E. coli DNA follows more or less the cooperative pattern of native DNA. This in other word means that sequence divergence among repeated sequences has not only decreased the melting temperature of reassociated calf thymus DNA but also have changed the pattern of melting. This may arise due to the fact that base mismatch is mainly distributed homogeneously along the sequence. Block sequence divergence is thus not prominent. Gel electrophoresis of repeated

sequences show that reassociated DNA do indeed form hyper-polymeric structure. The major portion of the reassociated DNA remains immobile at the top of the gel. Also important is the fact that the stability of the structures is comparable with the stability of the reassociated DNA. This is inferred from the fact that even at 70-75°C these structures retain their gel immobility in the presence of 1% formaldehyde.

The theoretical calculation of the reassociation concerted with the effect due to random breakage and length distribution predicted a more or less unchanged length distribution of the fragments during reassociation. Gel electrophoresis of unreassociated single strand DNA showed that upto 50% of the reassociation, the width of the DNA band remained unchanged - thus qualitatively verifying the prediction.

4) The fourth chapter deals with some problems in hydroxyapatite binding to DNA. Firstly reviewing the mechanism of binding of DNA to HAP the discussion pinpoints on the question - How does a duplex DNA elute out as single strand DNA at high temperature? Simple experiments designed to probe into the mechanism of ds to ss transition of DNA while bound to HAP shows that DNA binds to HAP in two discrete manners so far as this transition is concerned. This result is discussed in light of recent evidences on the possibility of left handed DNA existing along with its right handed counter part.