ABSTRACT

β-clamp has been known as a processivity enhancing factor for DNA polymerase III in *E. coli*. It has also been known to interact with other polymerases viz. DNA polymerase I, II, IV and V. Apart from these, β-clamp interacts with proteins involved in excision repair like MutS and DNA ligase. Okazaki fragment maturation requires the removal of the RNA primers efficiently and the ligation of the DNA to form an intact strand. These steps are extremely crucial for the survival of the organism and need to be done quickly and accurately.

To get deeper mechanistic insights, *in vitro* biochemical studies were conducted in this work involving the *E. coli* β-clamp, its clamp loader proteins, DNA polymerase I (Pol I) and DNA ligase. **Chapter I** summarizes the available information in literature regarding what is known about the *E. coli* β-clamp, its eukaryotic counterpart PCNA, the DNA polymerases and DNA ligase. An understanding regarding the nick translation property of DNA polymerase I has been provided, along with an overview of prokaryotic replication and a comparison between the replisomes in prokaryotes and eukaryotes. We also highlighted our objectives of the present work Chapter I.

Chapter II explains in detail the preparation of the necessary components and biochemical machineries used in the study. Cloning and purification of the proteins and their mutant counterparts have been explained elaborately. A due explanation has been given to the preparation of single stranded circular template to check the activity of β-clamp and its proven role in increasing the processivity of DNA polymerase I. Preparation of a gapped DNA to be used as a linear template to load β-clamp and study the implications it has on the polymerase and exonuclease activities of Pol I has also been elucidated in detail. Experimental confirmation of β-clamp loading on the template has been performed in this chapter too. Apart from these, standard techniques in molecular biology and bacteriology have been explained.

Chapter III explains the influence of β-clamp in regulating the functions of Pol I and DNA ligase. Experimental evidences proving the role played by β-clamp in inhibiting the nick translation property and strand displacement activity of Pol I has been explained. Experimental evidences proving the role played by β-clamp in increasing the ligation efficiency of DNA ligase have also been provided in this chapter. A complete protocol to determine the Relative Ligation Frequency has been provided. To

differentiate between the products formed due to polymerization and ligation, experimental details regarding the ligation assays have been provided, that elucidate clearly, how β-clamp enhances the ligation potential of DNA ligase by inhibiting the strand displacement and nick translation property of Pol I.

Chapter IV deals with the mechanistic detailing of the regulation of strand displacement and nick translation property of Pol I by the β-clamp. Experimental observations regarding the role of β-clamp in regulating the single nucleotide cleavage and flap cleavage by Pol I during nick translation has been studied elaborately. In addition, cross-linking assay to ascertain the impact β-clamp has on the conformation of the small domain of Pol I have been performed. Structural modelling to understand these conformational changes have been explained in this chapter.

Overall, the entire study elucidates the role of β-clamp in the strand displacement activity of Pol I apart from the reported role of enhancing the processivity of polymerases. This is the first time it is being reported that β-clamp has roles apart from merely increasing the processivity of the polymerases during DNA replication.