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DNA Repair Mechanisms and Mutagenesis

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I. INTRODUCTION

All living cells are constantly exposed to chemical and physical agents that have the ability to alter the primary structure of DNA. Such alterations, if not corrected, would result in mutations. While many of these mutations would be neutral (i.e., no changes in the amino acid sequences of peptides) or would be insignificant (no involvement of regulatory regions for the DNA and RNA or in the case of proteins no alteration of active sites), the accumulation of significant mutations has the potential to increase the genetic diversity of a species. Such genetic diversity is an essential component of evolution and the ability of species to survive in changing environments. However, there does come a critical point in the accumulation of mutations (genetic load) at which time the species can no longer exist (Dobzhansky, 1950). Thus it would seem obvious that living systems must maintain mechanisms for the repairing of DNA damage. It would also seem obvious that

these same systems must balance the removal of DNA damage with the accumulation of a finite number of mutations. In this chapter we discuss the diversity, as well as the exciting intricacy, of the DNA repair systems found in the paradigm *Escherichia coli*. In addition we consider the dramatic changes that have occurred within the last 10 years to our understanding of the processes of DNA repair and mutagenesis. Many of these changes have been brought about by the information gained through the various genome projects.

Beginning to understand the processes associated with DNA repair and mutagenesis requires visiting the debate over whether mutations arise spontaneously or are directed by environmental conditions—Darwin versus Lamarck. In 1942 Luria and Delbrück seemed to answer this question following the publication of their fluctuation tests (Luria and Delbrück, 1943). By combining statistics with

an elegant investigation of mutation numbers, these pioneers demonstrated that under their laboratory conditions bacterial mutations arose spontaneously during growth. While these and related results (Lederberg and Lederberg, 1952; Newcomb, 1949) clearly supported the view that mutations are non-directed and arise spontaneously, the debate has never really ended. The last 10 years has seen a dramatic increase in the interest shown in "directed" and stress-induced mutagenesis (Wright, 2000). While this is not a new concept, the very mention of naturally occurring "directed" mutagenesis invokes the passions associated with Lamarck's views on the inheritance of acquired characteristics. In all fairness, Lamarck should also be remembered for having articulated the need for a gradual evolution from the simplest species to the most complex. Evolutionists (Dobzhansky, 1950) and mathematicians have consistently questioned the probability that evolution could have proceeded as rapidly as demonstrated had true random mutagenesis been the only factor in providing genetic diversity (Wright, 2000). The validity of these questions is attested to, since today we know of the impact that transposons and transpositions can have on diversity and on the evolutionary process (Labrador and Corces, 1997; see Whittle and Salyers ch. 17). Furthermore there are data that strongly support the existence of stress-related "directed" mutagenesis mechanisms (Wright, 2000). However, it is important to note that in all of these cases there is no evidence found to support the Lamarckian concept of the inheritance of acquired characteristics.

Consistently spontaneous mutations were thought to arise almost exclusively as a consequence of growth (either errors in replication, unrepaired DNA damage or as a result of errors during the process of repairing damaged DNA). As described in the chapter by Frishein, this volume, prokaryotic DNA replication is the primary responsibility of the replicating complex. This complex of DNA polymerases and accessory proteins perform the normal semiconservative replication with

a great deal of accuracy (Friedberg et al., 2000; Friedberg et al., 1995; Ohashi et al., 2000). Without the involvement of any factors contributed by the bacteria, the potential error frequency associated with the pairing of bases would be between 1 to 10% per nucleotide. However, the actual mutation frequency for newly replicated *E. coli* DNA is six to nine orders of magnitude less frequent than the prediction based solely on energetics. At least three to six orders of magnitude of this enhanced fidelity is due to inherent properties associated with the replication machinery including the 3' to 5' exonuclease function that has editing or proofreading activity. Further reduction in the replication errors occur as a result of the functioning of a protein system involved in mismatch correction (described below).

Recently a family of error-prone polymerases that lack the 3' to 5' exonuclease editing function have been identified in eubacteria, archaea, and eukaryotes (Friedberg et al., 2000; Gerlach et al., 1999). In *E. coli* these designated DNA polymerases IV (DinB) and V (UmuD'YC) have been associated with translesion processing of DNA (replication past a noninstructive lesion) and consequently with the potential generation of mutations. In eukaryotes, homologs of these polymerases have been associated with human diseases including cancer and potentially with the functioning of the diversity associated with the immune system. The existence of these polymerases and their stress-related regulation has spawned an intensive re-investigation into the nature of the mutagenesis process(es).

In 1988 John Cairns and his collaborators published a controversial and exciting article that forced rethinking about how spontaneous mutations might arise when cells are under a stress-induced selection (Cairns et al., 1988). Although there were some problems with this first report (Prival and Cebula 1996), Cairns and Foster (1991) confirmed that mutations arise in nondividing or stationary phase bacteria when the cells are subjected to nonlethal selective pressure