STUDIES ON THE MOLECULAR MECHANISMS RESPONSIBLE FOR THE MAINTENANCE OF GENOMIC INFORMATION

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Ubiquitous environmental and endogenous genotoxic agents cause a myriad of lesions in DNA. These lesions interfere with normal DNA metabolism, including DNA replication, eventually resulting in mutations that can lead to carcinogenesis and/or cell death. To maintain the integrity of genetic material, cells possess multiple pathways, including checkpoint mechanisms that operate in various phases of the cell cycle and repair mechanisms, such as nucleotide excision repair (NER). However, not all lesions on the genome can be repaired efficiently by these processes in time for DNA replication, and some lesions are repaired very inefficiently. To prevent acute cell death through arrested DNA replication at unrepaired lesions, cells have acquired during evolution a variety of mechanisms that remove the impediment to replication through such lesions. These mechanisms are referred to as postreplication DNA repair.

In the course of working on the mechanism of chromatin replication in the cell-free SV40 minichromosome replication assay, we realized that this cell-free assay could be used to study the molecular mechanism of NER. We constructed a cell-free NER system with UV-irradiated SV40 minichromosomes and purified a protein complex that complements a repair defect in xeroderma pigmentosum group C (XP-C) patient cells. Subsequently, we identified cDNA clones encoding the three subunits of this complex, namely XPC, HR23 and Cen2. Biochemical studies revealed that the XPC complex is a structure-specific DNA binding factor that recognizes a change in DNA secondary structure rather than the DNA lesion itself. UV-damaged DNA binding protein (UV-DDB) is another complex that appears to be involved in the recognition of NER-inducing damage, although the precise role it plays and its relationship to XPC are still not clear. We found that XPC protein is polyubiquitylated in response to UV irradiation and that XPC ubiquitylation depends on the presence of functional UV-DDB. Both XPC and UV-DDB are polyubiquitylated by the recombinant UV-DDB-ubiquitin ligase complex and polyubiquitylation altered their DNA binding properties. The polyubiquitylation of XPC and UV-DDB is required for cell-free NER of UV-induced (6-4)photoproduct specifically when UV-DDB is bound to such lesions.

Among the eight XP complementation groups, XP variant (XP-V) remained an enigma for some time. Although it was realized early on that XP-V cells must have a defect in postreplication repair, it was first necessary to establish a cell-free system that could assay for so-called translesion DNA synthesis (TLS). By using this system, we purified a protein that could correct the TLS defect of XP-V cell extracts. The protein turned out to be a novel DNA polymerase that could bypass UV-induced cyclobutane pyrimidine dimer (CPD) and we named it DNA polymerase eta (Pol eta). Pol eta can bypass CPD efficiently and relatively accurately. Pol eta differs in its polymerase activity domain from previously identified polymerases and constitutes the first member of a new family of DNA polymerases, namely the Y-family. Y-family polymerases are found from prokaryotes to eukaryotes. Thus TLS is conserved evolutionary. Moreover, Pol eta is involved not only in TLS but also in somatic hypermutation and homologous recombination.