Published in final edited form as:

Trends Cancer. 2015 December 1; 1(4): 215–216. doi:10.1016/j.trecan.2015.11.001.

The journey of DNA repair

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Abstract

21 years ago, the DNA Repair Enzyme was declared “Molecule of the Year”. Today, we are celebrating another “year of repair”, with the 2015 Nobel Prize in Chemistry being awarded to Aziz Sancar, Tomas Lindahl and Paul Modrich for their collective work on the different DNA repair pathways.

The integrity of DNA is constantly under threat by many genotoxic agents both within and outside the cell. It is estimated that the DNA in a typical mammalian cell is exposed to 10^4 to 10^5 lesions every day in the form of altered bases, abasic sites, inter- or intrastrand cross-links, bulky adducts, mismatches and small insertions and deletions and single or double strand breaks [1]. Given the vast repertoire of damage encountered by the genome, it is hardly surprising that cells have evolved a multitude of repair pathways to maintain integrity. Either the absence or erroneous repair of such damage has dire consequences for the organism in the form of increased mutation load and chromosomal rearrangements. Unrepaired double strand breaks can also lead to loss of entire chromosomes. These phenomena have been implicated in cancers and various hereditary diseases. Examples include predisposition to hereditary non-polyposis colon cancer in individuals with defects in mismatch repair (MMR); colorectal cancer and sporadic glioblastomas associated with mutations in base excision repair (BER); breast cancer in carriers of mutations in *BRCA1* or *BRCA2*; Nijmegen breakage syndrome; cancer-prone ataxia telangiectasia; and Bloom and Werner syndromes due to defective recombination repair. Furthermore, increased mutation loads resulting from the lack of, or error-prone repair, have been proposed to play a role in aging. In support of this theory are several studies demonstrating that DNA damage and mutations accumulate in aging cells, as well as recent work linking defective DNA repair with premature aging phenotypes and an increased incidence of cancer [reviewed in [2]].

DNA repair – The early days

The discovery that DNA and its replication form the predominant basis of heritability triggered an avalanche of studies seeking to elucidate the factors that influence its stability.

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The knowledge of deleterious effects of mutagenic chemicals and radiation on DNA, accumulated mostly from classical genetics, soon led to the realization that repairing DNA lesions is critical. Amongst the first DNA repair pathways to be discovered was enzymatic photoreactivation - the process of reverting UV-induced pyrimidine dimers to monomers by photolyase in the presence of light. The cloning of the *E.coli* gene *phr* and its biochemical characterization by Aziz Sancar [3] was crucial in understanding the function of this remarkable enzyme. Thereafter, Sancar went on to study other mechanisms of DNA repair that are activated post UV exposure. His work along with others in the cloning and biochemical analysis of the bacterial *uvr* proteins steered the discovery of the nucleotide excision repair (NER) pathway [4]. Similarly, the 1974 discovery of DNA glycosylases in *E.coli* by Tomas Lindahl pioneered studies on the BER pathway [5]. Subsequently, nucleases dedicated to the cleavage and consequently repair of abasic sites formed in DNA after the action of glycosylases have been found in various other systems. Today, there are 11 known glycosylases in mammalian cells.

The existence of an enzymatic mechanism correcting abnormally paired bases in DNA was first postulated by Robin Holliday in 1964 to explain the fate of such mismatches formed in the process of meiotic recombination in ascomycetes [6]. It was later noted that MMR was also required to assure the fidelity of DNA replication by correcting nucleotide misincorporation and primer-template misalignment by the replicative polymerases. However, a key question remained unanswered – How is MMR able to distinguish between the parental and the newly synthesized strands of DNA? Using elegant biochemical and genetic approaches, Paul Modrich demonstrated that strand discrimination in *Escherichia coli* is achieved by differential methylation of the parental strand at adenines in the GATC context [7]. Modrich's subsequent work on both eukaryotic and prokaryotic MMR has been seminal in revealing the roles for the major players involved in this repair system.

DNA repair - The current era

The last few decades have witnessed a tremendous advancement in our understanding of DNA repair. Current technological breakthroughs have enabled us to address several unresolved questions regarding the intricate mechanistic details underlying various repair pathways: How is DNA damaged sensed by these pathways? Which are the key components of these pathways and what are their roles in repairing different lesions? Where in the cell do the different pathways operate? How do cells regulate error-free vs error-prone repair mechanisms? We now have valuable insight on a few of these problems, as illustrated briefly through the following examples:

The development of live cell imaging has led to the elucidation of the spatio-temporal interactions of repair proteins with DNA lesions in real time. Utilization of fluorescently tagged repair proteins with the ability to engineer damage at defined loci in the genome has enabled researchers to decipher the dynamics of DNA repair. Analogous approaches using fluorescent-tagging of double strand breaks in the genome have further revealed that broken chromosomes have increased mobility within the nucleus and that breaks tend to localize at the nuclear periphery during repair [8].

Another powerful approach gaining widespread popularity is the analysis of DNA repair at the single-molecule level, which allows researchers to stretch and visualize individual DNA molecules, and study the dynamics of DNA repair. This method was recently used to demonstrate that break-induced replication, a double strand break-repair pathway used to repair one-ended breaks, proceeds via a conservative mode of inheritance unlike the canonical semi-conservative DNA synthesis [9]. Other single-molecule techniques include the use of “DNA curtains” wherein individual DNA molecules are tethered at one end to help understand how proteins traverse the length of DNA; tethered particle motion to study the change in DNA length upon interaction with enzymes; and the use of molecular tweezers to study the mechanical properties of DNA-protein and protein-protein interactions [reviewed in [10]].

With the advent of the “omics” era it is now possible to analyze the mutagenic potential of various endogenous and exogenous mutagens and to study repair efficacy across the genome. Chromatin-immunoprecipitation (ChIP) using antibodies against lesions, in combination with Next-Gen sequencing, has been extremely valuable in determining the global pervasiveness of damaging lesions such as RNA:DNA hybrids [11]. ChIP has been additionally used with antibodies against various DNA repair factors in order to measure their accessibility to different genomic regions. Additionally, high-throughput sequencing of cancer genomes has immensely helped in detection of the mutational mechanisms operative in them. For instance, cancers with defective mismatch repair demonstrate elevated levels of microsatellite instability. On the other hand, increased clusters of A/T mutations have been attributed to the translesion polymerase Pol η and this signature is detectable in some blood cancers, such as multiple myeloma, chronic leukemia and B-cell lymphoma. Furthermore, evidence of impaired repair can be gleaned from the elevated amount of mutations attributable to known mutagens. Notable examples include UV-induced C $\rightarrow$ T changes in TC or CC contexts or A:T $\rightarrow$ T:A mutations induced by mutagenic aristolactam adducts formed by aristocholic acid that are repaired by NER [reviewed in [12]].

Conclusion

The availability of cutting edge technology has ushered in a new era of DNA repair research where studies from model organisms serve as a basis to elucidate the mechanisms underlying repair in complex systems including human cancers. The future holds great promise for the field as we continue to seek better understanding of the cellular mechanisms employed to preserve genome integrity. This is truly a great time to be in the field of DNA repair!

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