

Bacteria and DNA repair – 50 years together

Peter Strike

From the discovery of the double helix it has taken 50 years of intense study to understand DNA repair. Nearly all key breakthroughs have come from work with bacterial cells.

DNA repair now sits centre stage, along with DNA replication and the cell cycle, as one of those key processes essential to the function of every living cell. It is of course inaccurate to describe it as a single process – it is a comprehensive suite of functions, able to deal with almost every environmental challenge thrown at our DNA, maintaining the molecule's structure and genetic integrity. Defects in repair impact on survival, mutation, cancer, antibody production, reproduction, meiosis, mitosis ... the list is endless. And yet, not too long ago, DNA repair was considered an odd little corner of biology, largely studied in a rather oblique manner by the 'radiobiologists'. The subject was generally outside mainstream interests, and indeed it could be considered that a general realization of the importance of repair processes really only came with the demonstration of the unequivocal link between mismatch repair and colon cancer in the 1980s. Almost no biochemical analysis of repair functions was even attempted until the 1960s, although good genetics had begun to indicate the presence of these processes somewhat earlier.

was reporting the mutagenic effects of ultraviolet light on micro-organisms, and indeed reporting that that the optimum wavelength for mutation coincided with the peak of absorption for nucleic acids. Doubt, however, was still prevalent that DNA could be an important molecule. Max Delbrück, for example, is quoted as acknowledging that while much evidence pointed to DNA as the genetic material, 'it was believed that DNA was a stupid substance, a tetranucleotide which couldn't do anything specific'.

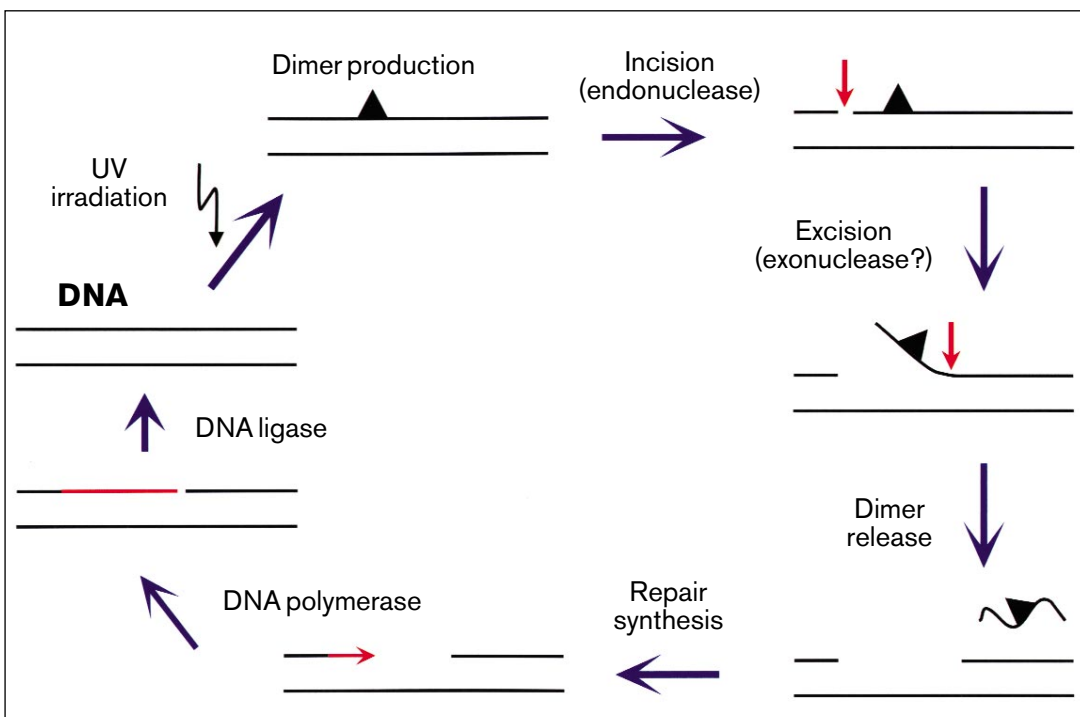
What began to change things was of course the discovery of that structure, the beautiful double helix that suggested to its discoverers not only 'an obvious mechanism for its replication', but also a mechanism for spontaneous mutation 'due to a base occasionally occurring in one of its less likely tautomeric forms'. The possibility of DNA repair was not immediately inferred from the structure, but became current in the early 1960s from the work of Bob Haynes and others as a theoretical explanation for a variety of phenomena including 'liquid holding recovery', in which it was observed that cells (of bacteria or yeast), if held in starvation medium for increasing periods following irradiation, showed remarkable levels of recovery. By 1964, Haynes and Philip Hanawalt were sufficiently sure of their ground to point out that the redundancy inherent in the double helix provided a means by which, if one strand of the DNA helix was damaged, 'the information in that portion could be retrieved from the complementary strand'. Such thoughts were current in a number of laboratories and, from one of these (Dick Setlow's), a biochemical description of the first repair pathway to be understood at this level was about to appear. That pathway was nucleotide excision repair.

● Early discoveries

The study of repair started as a consequence of the fascination in the early 20th century with the biological effects of radiation. Herman J. Muller's pioneering work, generating mutants of *Drosophila* with X-rays, led to a more general interest in radiation of all kinds, with UV becoming a firm favourite because of its ease of use and considerable efficacy. By 1941, Alexander Hollaender

BELOW:

Fig. 1. Typical 1960s model of nucleotide excision repair, based on the observations of P. Setlow, P. Howard-Flanders, P. Hanawalt and others. Assignment of enzymes to particular steps is speculative, but the overall picture of events proved to be remarkably accurate. COURTESY P. STRIKE



● Repair in microbes

The Haynes and Hanawalt suggestion has to be set in the context of the elegant genetic and physiological work, done primarily with bacteria and bacteriophages, but also with eukaryotic micro-organisms, which showed beyond doubt that DNA repair was occurring. And more, much more than this – the bacteria did it their way! First, there was light-dependent repair, or photo-reactivation, discovered by Albert Kelner in 'the fungus'

Streptomyces griseus. Then excision repair, discovered initially through the phenomenon known as 'host-cell reactivation', in which the damage done to UV-irradiated bacteriophages could be repaired upon infection into repair-proficient bacterial cells. The demonstration that host-cell reactivation was truly due to a repair process required one key ingredient – a mutant bacterial

strain in which repair was defective. This key ingredient came from the laboratory of Ruth Hill, with her isolation of the UV-sensitive bacterial strain *Escherichia coli* B_{S-1}. This strain, some 100 times more sensitive to UV light than the parental strain, provided the vital tool with which to investigate the putative process of excision repair, allowing as it did a direct comparison at the biochemical level of the differences between mutant and wild-type parent. Many labs had by this time predicted how excision repair might work, but it was Dick Setlow who provided the proof, demonstrating that damage-containing oligonucleotides were released from high-molecular-mass DNA in wild-type *E. coli*, but not in the sensitive B_{S-1} strain.

● *E. coli* leads the field

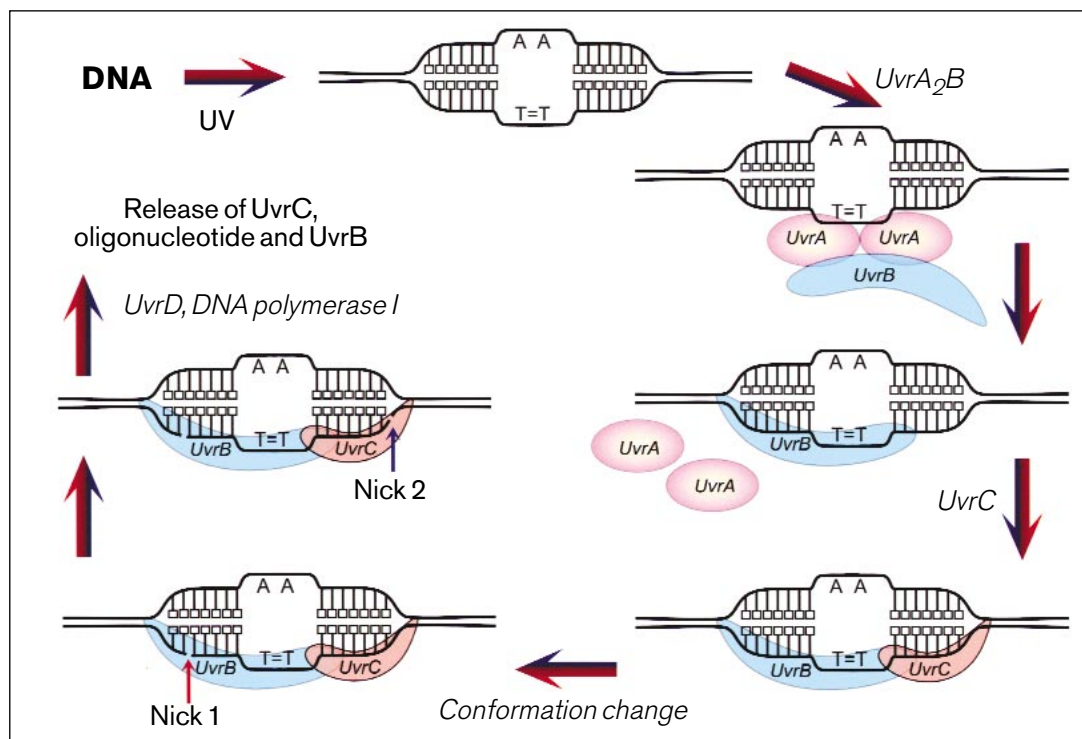
Discoveries from *E. coli* now came thick and fast. Paul Howard-Flanders' group isolated the excision-repair defective *uvr* mutants in *E. coli* K12, allowing a thorough genetic analysis to be undertaken. Dean Rupp and Howard-Flanders showed that a second type of dark repair, termed post-replication repair, occurred in excision-repair-defective mutants. Mutants defective in recombination were isolated by John Clark, and shown to be defective in this newly identified repair process. Mismatch repair was discovered, this elegant mechanism exploiting delayed methylation of newly replicated DNA to direct repair of mismatched bases to the newly synthesized strand. The concept of excision repair was extended to base excision repair (removal of a single damaged base rather than an oligonucleotide), a mechanism involving different initiating enzymes from those identified in the *uvr* mutants, but sharing many later steps. The *uvr*-based mechanism was now renamed 'nucleotide excision repair' to distinguish it from this new mechanism. Studies of mutation resulted in the isolation of a number of classes of 'non-mutable mutants', confirming that mutation too was an enzyme-mediated process, and culminating in the proposal of error-prone trans-lesion synthesis put forward by Evelyn Witkin and Bryn Bridges. In each of these cases, we now

have flesh added to the bones. Base excision repair includes a whole set of mechanisms for the removal of different types of damaged (or incorrect) bases from DNA. Why is there no uracil in DNA? Because base excision repair removes it. Mismatch repair is also multifaceted, a family of processes recognizing different types of mismatched bases and of such importance that closely related enzymes are conserved from bacteria to man. A highlight of the 1980s was the isolation of the three components of the *uvr* excision repair nuclease by Aziz Sancar and Dean Rupp which caused the journal *Science* to nominate DNA repair enzymes as 'molecule of the year' in 1984. Equally stunning was the elucidation of the genetics and biochemistry of the 'SOS response', the environmentally responsive system controlling the expression of many DNA repair and recombination enzymes. A second controlled system, identified initially by John Cairns as the 'adaptive response', allowed the *E. coli* cell to respond to methylation damage. Characterization of this system resulted in the identification of the alkyltransferases, proteins (not strictly enzymes) that could remove alkylation damage from DNA by transferring it to themselves and which, in the case of the Ada protein, also played a key role in the control of the response.

● Recent developments

And what has the last 20 years brought us? The concepts of transcription-coupled and strand-specific repair, removing damage preferentially from active genes. A recognition of the importance of oxidative damage, particularly that generated by normal cellular processes. A detailed understanding of many of the enzymes of DNA repair and recombination – the sheer beauty of the branch migration process mediated by RuvABC, and the protein helix formed by RecA as it coats DNA. The unexpected, but perhaps predictable, discovery that *umuDC* gene products, defects in which abolish UV-induced mutation, in fact encode an error-prone DNA polymerase capable of trans-lesion synthesis.

But, perhaps most importantly, they have brought the



ABOVE: Fig. 2. A simplified model of the incision events of nucleotide excision repair, as we currently understand them. Specific roles for the Uvr proteins have been identified, and the principal difference from the predictions shown in Fig. 1 is that the Uvr enzyme complex actually makes co-ordinated strand breaks at precise positions on both sides of the damage site. The presence of damage is initially detected by a UvrA₂B protein complex scanning DNA for distortions. The UvrB protein is then loaded at the damage site, in a transient complex from which the two UvrA molecules are rapidly lost. The very stable UvrB/damaged DNA complex then attracts one molecule of UvrC to create an incision complex. Recruitment of UvrC results in activation of UvrB to make one single-strand nick 5 bases 3' to the damage. A further change in conformation activates UvrC to make a second nick 7 bases 5' to the damage. Subsequently, the combined action of UvrD protein and DNA polymerase I results in the release initially of UvrC, then the damage-containing oligonucleotide, and then UvrB. Repair synthesis then refills the gap so created. The model makes no attempt to show the bend induced in DNA by interaction with the repair proteins, nor does it show any of the interactions with other proteins that direct repair preferentially to active genes. COURTESY P. STRIKE

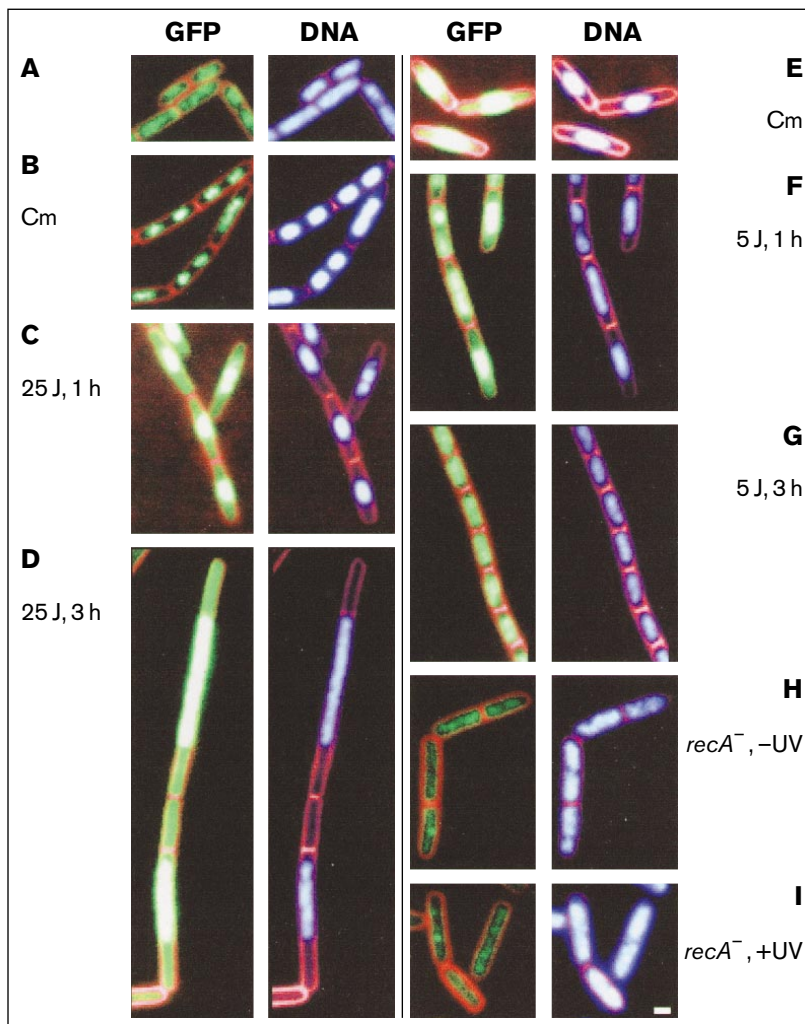
RIGHT:

Fig. 3. Responses of *Bacillus subtilis* to DNA damage. The GFP (green)-labelled protein in the left-hand column is UvrA, a key component of the excision repair mechanism. DNA is stained with DAPI (blue) (right-hand column), and cell membranes with dye FM4-64 (red). Panel A shows normal growing cells, not exposed to any exogenous DNA damaging agent. It is clear that the DNA fills most of the cell volume available, and that UvrA protein is associated with DNA even under undamaged conditions. In panel B, the cells have been treated with chloramphenicol, to inhibit protein synthesis. Under these conditions, initiation of DNA replication is inhibited, and the nucleoid adopts a more compact appearance. The UvrA protein is still clearly associated with the DNA. Similar compaction of the nucleoid is seen if the cells are exposed to UV irradiation (panels C and D). UV also induces higher level expression of the *uvrA* gene, resulting in high levels of protein. Cell size increases with time, but the nucleoid fails to fill the space available, and some cells are created that contain no DNA whatsoever. UvrA protein remains tightly associated with the DNA in those cells that contain it. Similar compaction of the nucleoid is observed when cells are treated with the DNA cross-linking agent mitomycin C (panels E and F). At lower UV doses, nucleoid compaction is observed after short incubation times (panel F), but is effectively reversed as repair of DNA damage is completed (panel G). The mechanisms underlying nucleoid compaction are not well understood, but appear to require induction of the SOS response. Compaction is not observed in a *recA* mutant, which cannot induce the SOS response, although association of UvrA with DNA is still clearly maintained (panels H and I).
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realization that DNA repair and DNA recombination do not go on in isolation. They are key cellular processes, integrated with other major aspects of cell function, including cell division, DNA replication and transcription. We now know that a damaged cell stops cell division and DNA replication, until DNA repair has removed the damage. Indeed the bacterial cell seems to radically alter the structure of the nucleoid, so that repair can proceed more efficiently. Should a replication fork hit a damaged site and stall, the replication fork may go into reverse, to generate a ‘chicken-foot’ structure which may then be processed to allow replication to proceed across the damaged site. A similar response may occur if a replication fork hits an RNA polymerase which has stalled at the site of DNA damage.

● What next?

From the discovery of the magic double helix, it has taken 50 years of intense study to come to an understanding of DNA repair that was not, indeed could not be, imagined at the time. Almost all of the key breakthroughs have come from studies on bacterial cells, with an honourable mention for the eukaryotic microbes, particularly *Saccharomyces cerevisiae*. There are undoubtedly more surprises to come. We have yet to explain the extraordinary radiation resistance of the most resistant of all bacteria, *Deinococcus radiodurans*, which is capable of reassembling an intact chromosome following complete fragmentation by exceptionally high doses of ionizing radiation. A new link has become apparent here, in that radiation resistance and resistance to dehydration/rehydration clearly depend on common processes in this organism. All in all, that is not a bad record for a molecule once considered too stupid to be of any interest but which, once its true structure was revealed, became central to all biological thinking.



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