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Historical Perspective on the DNA Damage Response

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Abstract

The DNA damage response (DDR) has been broadly defined as a complex network of cellular pathways that cooperate to sense and repair lesions in DNA. Multiple types of DNA damage, some natural DNA sequences, nucleotide pool deficiencies and collisions with transcription complexes can cause replication arrest to elicit the DDR. However, in practice, the term DDR as applied to eukaryotic/mammalian cells often refers more specifically to pathways involving the activation of the ATM (ataxia-telangiectasia mutated) and ATR (ATM-Rad3-related) kinases in response to double-strand breaks or arrested replication forks, respectively. Nevertheless, there are distinct responses to particular types of DNA damage that do not involve ATM or ATR. In addition, some of the aberrations that cause replication arrest and elicit the DDR cannot be categorized as direct DNA damage. These include nucleotide pool deficiencies, nucleotide sequences that can adopt non-canonical DNA structures, and collisions between replication forks and transcription complexes. The response to these aberrations can be called the *genomic stress response* (GSR), a term that is meant to encompass the sensing of all types of DNA aberrations together with the mechanisms involved in coping with them. In addition to fully functional cells, the consequences of processing genomic aberrations may include mutagenesis, genomic rearrangements and lethality.

Keywords

DNA damage response; DNA repair; SOS response; Genomic stress response; ATR ATM

1. Introduction

The proliferation of living cells requires that the genomic DNA must be replicated. A proliferating cell must duplicate its entire complement of DNA with astonishing precision in the face of a barrage of deleterious endogenous and environmental genotoxic agents, as well as the intrinsic chemical instability of the DNA molecule itself [1]. Naturally occurring non-canonical DNA structures can also pose a challenge to replication [2, 3]. Transcription complexes, translocating along the same DNA track, may collide with replication forks [4].

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Conflict of Interest Statement

The author declares that there are no conflicts of interest.

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Some types of encumbrances are more critical than others; one double-strand break or an interstrand crosslink can, in principle, be sufficient to preclude the generation of viable daughter cells [5]. It is not surprising that a complex set of responses has evolved within the past four billion years to deal with all types of damage and other obstructions that might prevent completion of the DNA replication cycle and the allocation of essentially identical genomes to progeny. Somewhat less important to cell proliferation are the consequences of most mutations, even though a subset of those will also impact the essential processes of replication and completion of the cell cycle.

The primary approach to minimize mutagenesis and to ensure completion of genomic replication is to repair the offending DNA lesions. Several of the DNA repair pathways (e.g. base excision repair (BER) and those initiated by ATM-Rad3-related (ATR) are so important that life cannot be sustained without them (cf. [6]). Furthermore, there are a number of hereditary diseases with predisposition to cancer and/or aging that are linked to deficiencies in DNA repair. These are detailed in numerous reviews and several texts in this rapidly developing field (cf. [7–11]). The various DNA repair pathways sometimes compete with each other for processing the same lesion, and each step in a multistep repair pathway creates an intermediate that constitutes another lesion (e.g. strand break or single-strand gap), which may be susceptible to intervention by enzymes from another pathway. The overall response to DNA damage may be viewed as a set of successive stages, with a decision point at each stage until the DNA integrity has been restored [12]. Sometimes the first protein to access the lesion may be a transcription factor or another protein that is not directly involved in DNA repair. The outcome for the cell, and the organism of which it is a part, may depend upon which protein first encounters the lesion [13]. Also, the response to the damage may require a threshold level of damage so that very low levels of lesions might be overlooked, whereas substantial amounts of damage or particular types of lesions (e.g. double strand breaks) may induce a robust response. Thus, the overall DNA damage response consists of many separate and sometimes competing components, and the outcome for the cell may not necessarily be ascribed to a particular pathway without full knowledge of the growth phase and cellular environment; for example, it is particularly important to know whether the cell is undergoing DNA replication, or is in a quiescent or terminally differentiated state. The comprehensive response could be termed the genomic stress response (GSR) to accommodate those situations in which there is no initial damage, even though one or more of the processing steps may generate damage in the course of the response. The cellular outcome, in terms of mutagenesis or lethality, is clearly a downstream event that is dependent upon many factors following the initial recognition of an aberration.

2. Early history of DNA damage responses

Photoreactivation was probably the first example of an elucidated DNA damage response (see recent review [14]). In the early 1960s photoreactivation was shown to require a photolyase, which binds UV-induced cyclobutane pyrimidine dimers (CPDs) and upon activation by visible light, reverses them without breaking phosphodiester bonds in the DNA backbone. Photoreactivation is arguably the first DNA repair mechanism to have evolved, since it was likely essential for the survival of early life forms under the intense UV flux from sunlight, before there was a protective ozone layer in the stratosphere. Of course, the

shorter UV wavelengths would have continued to generate CPDs while photolyase was attenuating them, so the resulting steady-state level of these lesions must have been an ongoing threat. Although CPDs were not identified until 1960 [15], evidence for mechanisms to deal with UV-induced damage in the dark arose from the phenomenon of liquid holding recovery; the survival of UV-irradiated bacteria was enhanced (and mutagenesis was suppressed) upon nutrient deprivation for a period following the irradiation, later considered to provide a “window” for repair while other DNA transactions were suppressed [16–19]. The idea of “dark repair” was also supported by the isolation of mutants affecting UV sensitivity of bacteria [20–25]. The ubiquitous pathway of nucleotide excision repair (NER), discovered in 1964 in *E. coli*, utilizes the redundant genetic information in duplex DNA. A stretch of nucleotides containing a CPD or other lesion in one strand can be excised, and the resulting gap can be filled by repair replication using the intact complementary strand as template [26–28]. Polynucleotide ligase, discovered in 1967 [29], joins the newly-synthesized patch to the contiguous parental DNA strand. Evidence for NER in humans was initially reported as “unscheduled DNA synthesis” following UV irradiation of non-S-phase cells [30], and later validated as repair replication [31]. In the course of evolution some organisms, including humans, have lost the capability for photoreactivation to eliminate CPDs, evidently in favor of NER, which is highly efficient, more versatile and not constrained by requiring sunlight. The excision-repair modes of BER and mismatch repair (MMR) were reported in the mid-1970’s [32, 33].

With respect to excision-repair, early concern was raised about the complication created if an advancing replication fork encountered the lesion site following the excision step but before completion of the repair patch. The likely outcome, illustrated in Figure 1, is a complicated sort of double-strand break (with only one duplex end), which probably would be lethal. A bacterial culture in which DNA replication had been synchronized by the inhibition of protein and RNA synthesis to allow completion of the cycle without new initiations was strikingly resistant to UV in comparison to an asynchronous culture in exponential growth. In contrast, the NER deficient strain, *E. coli* B₅-1, was equally sensitive to UV during exponential growth or when the DNA replication cycle had been completed, implying that NER was essential for the enhanced resistance in the wild-type cells [34]. Repair replication was documented at the restrictive temperature in several temperature-sensitive strains of *E. coli*, unable to carry out chromosomal replication at the restrictive temperature [35]. Nearly identical UV survival curves were reported for *E. coli* strain TAU irradiated in stationary phase or after starvation for the required arginine and uracil [36]. It was concluded that the remarkable UV resistance of stationary phase cells results from the completion of DNA repair in the absence of chromosomal DNA replication; this emphasizes the importance of completing repair to avoid collisions of replication forks with lesions or intermediates in their repair [34].

3. Inducible responses to DNA damage and replication fork arrest

The enzymes required to detect damaged DNA can sometimes attack undamaged DNA, and such gratuitous events might be deleterious. (Any DNA strand break potentially puts the cell at risk.) A hierarchy of NER activity in extracts of *E. coli* and human cells was shown to act on various lesions with different affinities, and this included “undamaged DNA” at a low

level [37]. The situation is also complicated by the fact that some natural DNA sequences can adopt non-canonical secondary structures that can appear as DNA lesions to the lesion recognition enzymes. Thus, it makes sense that cells might maintain these enzymes at relatively low concentrations, unless there are significantly high levels of DNA damage. This issue is encapsulated by the title of an exceptional review by Ciccia and Elledge, “The DNA damage response: making it safe to play with knives” [38]. In the *modern version* of liquid holding recovery, the cell cycle checkpoints activated through the DDR provide a window of opportunity for DNA repair, before active replication forks and other DNA transactions are permitted.

Early evidence for an inducible response to UV-induced DNA damage was the phenomenon of UV-reactivation (also termed Weigle reactivation), by which the survival of UV-irradiated bacteriophage lambda was markedly increased if the host cells were also irradiated [39]. Photoreactivation of the host cells prior to the viral infection eliminated the phenomenon, but until 1960 we didn’t know what UV did to DNA, so the mechanism was obscure. Evelyn Witkin’s studies provided the first indication of an inducible DNA repair activity related to UV-reactivation in bacteria [40–42] and this inducible genomic stress response was confirmed, elaborated and named the “SOS response” by Miroslav Radman [43, 44]. The arrest of DNA replication in *E. coli* is now known to induce expression of a large number of genes with roles in genomic maintenance and control of cell division; including the up-regulation of *uvrA* and *uvrB*, required for lesion recognition in NER (cf. [45–47]). Elimination of the SOS response genetically or by treatment of the bacteria with rifampicin to prevent transcription, strikingly reduces the efficiency of CPD repair [48], but the more structurally distorting 6-4 photoproduct is easily recognized and repaired without up-regulation of the NER enzymes. The results obtained when p53 is activated through the ATR pathway following UV-irradiation in human cells are remarkably similar to those observed with the SOS system in *E. coli*. Although the 6-4 photoproducts are efficiently repaired, CPDs are poorly repaired unless p53 is activated; the mechanism involves the p53 dependent up-regulation of DDB2 (XPE), an accessory lesion recognition enzyme, which then recruits XPC, the primary enzyme for initiating the global genome repair (GGR) sub-pathway of NER [49–51]. These findings are in accord with the view that the highly sensitive DNA damage recognition enzymes are kept in check at low concentrations or otherwise constrained until they are needed to deal with specific types and levels of DNA damage.

The arrest of transcription by lesions or other encumbrances can be just as serious for cell viability as the blockage of replication. Persistent arrest of transcription triggers a strong signal for apoptosis, and the stalled transcription complex poses a challenge for an approaching replication fork [52, 53]. The sub-pathway of NER, termed transcription-coupled repair (TCR), is targeted to lesions in the transcribed strands of expressed genes (for reviews see [54–56]). The mechanism of lesion recognition by a translocating RNA polymerase is quite distinct from that for GGR, which involves a sensing of the stability of the DNA duplex structure [54]. Some types of damage are invisible to GGR but efficiently sensed for repair by TCR. The adenine adducts of the aristolactam metabolite of aristolochic acid are recognized for repair only in the template strands of expressed genes [57]. The

adduct 3-(deoxyguanosin-N²-yl)-2-acetylaminofluorene, blocks transcription and is removed exclusively by TCR [58], and the DNA adducts produced by acylfulvene and illudin S are also selectively repaired by TCR [59, 60]. Unlike GGR, TCR is constitutive and evidently not inducible through the DNA damage response.

Whereas the excision-repair pathways remove lesions to restore DNA integrity, there are tolerance pathways for enhancing survival by helping replication to overcome replication blocking lesions or other obstructions without removing them. Although these pathways are sometimes error-prone the offending lesions may be repaired later, hopefully before being encountered by another replication fork. While some of the tolerance pathways are inducible others are constitutive. They include the recruitment of specialized DNA polymerases, which can carry out translesion synthesis upon substitution for the blocked replicase [61, 62]. Some but not all of the translesion polymerases require activation through a DDR. In *E. coli* the concentrations of DNA polymerases II, IV and V (the main translesion polymerase) are increased through the SOS response, while the replicative and repair DNA polymerases, Pol I and Pol III are constitutive. The relative concentrations of Pols II, III, IV and V appear to control their access to the replication fork simply by mass action. In contrast, the expression of the mammalian translesion DNA polymerases is relatively constant and their activities are regulated by intracellular localization (in replication factories) and by access to the replication fork, determined by ubiquitination of PCNA. At the same time the ATR checkpoint activation at stalled forks includes the phosphorylation of Rev 1 and Pol eta. Finally, there are post-replication recombinational responses, of which some are constitutive while others are inducible. The pathways of genetic recombination are essential for dealing with double strand breaks and for the “fork collapse” that can occur when the fork encounters a single-strand break in the leading strand side of the template DNA. Particularly in eukaryotic cells, the pathways include the error-prone non-homologous end-joining pathway and variations of this mode, as well as the homologous recombination pathway, which requires that there be an intact DNA molecule with which the recombination can be completed, generally in an error-free manner [63]. A “collapsed replication fork” is operationally defined as a fork that has lost the capacity to perform DNA synthesis, a condition that can result from a number of different conditions in addition to DNA damage [64]. While fork collapse is rare in normal cells upon treatment with DNA damaging agents it is strikingly increased in cells lacking the ATP-dependent checkpoint and its downstream effector kinase CHK1 [65]. However, the Chk1 regulated ubiquitination of PCNA to facilitate translesion synthesis requires Claspin, but evidently not ATR, so Chk1 and Claspin operate to protect stressed replication forks independently of ATR [66].

4. The impact of chromatin on the DDR

In early years we overlooked the likely complications imposed by chromatin structure in eukaryotes with respect to access of damaging agents to DNA, and the access of repair enzymes to DNA lesions. We now know that chromatin can protect DNA from oxidative damage c.f. [67]. The analysis of the interactions of DNA excision-repair with chromatin was pioneered by Cleaver [68] and in the group of Michael Lieberman by Michael Smerdon, Thea Tlsty and Michael Kastan [69–72]. Higher levels of repair replication were measured in nuclease sensitive regions than in resistant regions of chromatin in UV irradiated human

cells, and nucleosome rearrangement in mammalian chromatin was documented during repair [69]. It was later shown that UV-induced 6-4 photoproducts were largely restricted to the inter-nucleosome regions, whereas CPDs were more uniformly distributed in chromatin. Treatment with sodium butyrate, causing hyperacetylation of the core histones, stimulated DNA repair in UV-irradiated human cells [73]; for review see [74]. The article by Bakkenist and Kastan on chromatin perturbation during the DNA damage response and other articles in this special issue provide information on current understanding in this rapidly developing area in the DNA repair field.

5. Revisiting the definition of the DDR

The DNA damage response in *E. coli* is clearly less complex than that in eukaryotes, in part due to the lack of chromatin structures, although some basic aspects are similar. In making comparisons it is useful to distinguish between the response to stalled replication forks and that due to other types of genomic stresses. The activation of RecA and the initiation of the SOS response, through interaction with the single-strand binding protein and the single strand regions that appear at arrested replication forks in *E. coli* is analogous to the activation of ATR upon binding RPA-coated single strand DNA at stalled forks in mammalian cells. The ssDNA-RPA complex at an arrested fork recruits ATR through its regulatory subunit ATRIP [75], and loads the PCNA-related complex 911 [76]. Additionally, the DDR can be activated by the operation of NER, particularly in situations in which the post-incision steps are inhibited (e.g. DNA polymerase inhibition) and excision gaps are extended. As with arrested forks, the mechanism involves stretches of single strand DNA coated with RPA, to trigger ATR-dependent signaling. (For current model see [77]. Thus, the activation of ATR can be triggered by some events that do not constitute damage, although these may all be related to the generation of single strand DNA regions, if not the arrest of replication (c.f. [78, 79]). ATR is associated with the nuclear envelope during S-phase of the cell cycle, where it can be activated by osmotic stress and mechanical stretching. It then relocates to the nuclear membranes, independently of cell cycle stage or DNA damage [80]. Tresini et al [81] report that transcription-blocking lesions result in R-loops that can activate ATM and displacement of active spliceosomes to activate non-canonical ATM signaling and modulate pre-mRNA splicing. ATM can also be activated by osmotic shock and by chloroquine, neither of which cause DNA damage [82].

Some types of lesions are recognized and repaired directly without the evident involvement of either ATR or ATM in mammalian cells, or the SOS response in *E. coli*. The pathways include those initiated by photolyase (discussed earlier) and the alkyltransferases and dioxygenases, which recognize their substrates directly and flip the damaged base out of the DNA duplex to reverse the damage (for review see [83]). The adaptive response to alkylation damage (e.g. O⁶-methyl guanine) in *E. coli* is independent of the SOS response and includes transcriptional induction of genes for direct reversal as well as BER. The constitutive levels of BER and NER are sufficient for dealing with low levels of their substrate lesions, and the principal signal for up-regulation of these pathways is the blockage of replication forks at lesions (For review see [84]).

There is also an ATR-independent DNA replication checkpoint, that remains intact even when both ATR and ATM are knocked out, as revealed from studies in primary mouse cells [85].

The term DDR is well established as the definition of the cellular responses to DSBs. Thus, Lemaitre and Soutoglou state: “To cope with damage the appearance of a DSB activates the DNA damage response (DDR) – a complex network of processes that allows recognition of the break and the activation of checkpoints, allowing the coordination between cell cycle progression and DNA repair” [86]. A broader definition is offered by Derks et al.: “All DNA repair systems, cell cycle checkpoints and additional pathways *whose activity changes upon DNA damage* are collectively known as the DDR.” [87]. However, there are responses for which the constitutive activity of particular enzymes and pathways is sufficient and more direct. These include the deployment of some translesion DNA polymerases at replication forks when the replicase is arrested at a lesion, as discussed in section 3 above.

Figure 2 represents an attempt to display all of the types of damage or hurdles for DNA replication in one comprehensive diagram. Although broad features and substrates for the respective pathways are indicated, there are many examples of cross-talk between pathways that would be difficult to include in one simple figure.

In conclusion, those who use a narrow definition of the DDR should continue to do so, because the selected group that follows that literature is fully aware of the limitations of this definition. However, the authors should always indicate in their Abstract and Introduction the nature of the DNA alteration and/or cellular treatment even if, by damage, they mean DSBs. For students and others who are just entering the field of genomic maintenance, it would be advantageous to have a broader definition, called the genomic stress response (GSR), which can accommodate the entire spectrum of genomic alterations and their processing, including those that involve induction of enzyme activities and post-translational modifications, as well as those that deal directly with DNA alterations. The ultimate purpose of the GSR is to ensure that living cells can complete the DNA replication cycle and cell division that are essential for their proliferation.

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Figure 1.

Early model for the expected consequence if a replication fork encountered a single strand break in the DNA template strand. This was presented after the discovery of repair replication but prior to the discovery of Okazaki fragments and discontinuous lagging strand synthesis.

Adapted from P. Hanawalt, "The UV sensitivity of bacteria; its relation to the DNA replication cycle" *Photochem. Photobiol.* 5: 1–12 (1966).

THE GENOMIC STRESS RESPONSE (GSR)

Figure 2.

Examples of threats that may initiate a genomic stress response (GSR) and some modes for their processing, with deleterious consequences or undamaged cells. Base damage includes alkylation, oxidation and deamination (e.g. cytosine to uracil), repairable by BER, although also some alkylations are reversible by alkyltransferases or dioxygenases. Abasic sites and strand breaks are intermediates in BER, but abasic sites are also generated by spontaneous depurination. Mismatch repair deals with errors during replication and small loops (that can arise through strand slippage). CPDs and bulky adducts such as polycyclic aromatic hydrocarbons (PAH) are repaired by NER. Non-canonical DNA structures are also subject to processing by repair systems even though formed in natural DNA sequences. Transcription may be arrested at lesions or unusual DNA structures to require processing by transcription-coupled repair (TCR). A translocating or blocked RNA polymerase may be encountered by a replication fork, requiring special processing. Double strand breaks (DSB) or interstrand cross-links (ICL) are the most serious types of damage, usually requiring several repair pathways in concert for their restitution, and inevitably resulting in activation of the GSR. A single strand break is a serious threat to a replication fork, but also to a transcription complex. The outcomes from processing these multiple threats require intricate regulatory systems, which include the cell cycle checkpoints triggered by ATM and ATR activation.