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Nucleotide excision repair "a legacy of creativity"

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Abstract

The first half of the 20th century has seen an enormous growth in our knowledge of DNA repair, in no small part due to the work of Dirk Bootsma, Philip Hanawalt and Bryn Bridges; those honored by this issue. For the new millennium, we have asked three general questions: (A) Do we know all possible strategies of nucleotide excision repair (NER) in all organisms? (B) How is NER integrated and regulated in cells and tissues? (C) Does DNA replication represent a new frontier in the roles of DNA repair? We make some suggestions for the kinds of answers the next generation may provide. The kingdom of archea represents an untapped field for investigation of DNA repair in organisms with extreme lifestyles. NER appears to involve a similar strategy to the other kingdoms of prokaryotes and eukaryotes, but subtle differences suggest that individual components of the system may differ. NER appears to be regulated by several major factors, especially p53 and Rb which interact with transcription coupled repair and global genomic repair, respectively. Examples can be found of major regulatory changes in repair in testicular tissue and melanoma cells. Our understanding of replication of damaged DNA has undergone a revolution in recent years, with the discovery of multiple low-fidelity DNA polymerases that facilitate replicative bypass. A secondary mechanism of replication in the absence of NER or of one or more of these polymerases involves sister chromatid exchange and recombination (hMre11/hRad50/Nbs1). The relative importance of bypass and recombination is determined by the action of p53. We hypothesise that these polymerases may be involved in resolution of complex DNA structures during completion of replication and sister chromatid resolution. With these fascinating problems to investigate, the field of DNA repair will surely not disappoint the next generation.

Keywords: Nucleotide excision repair; Xeroderma pigmentosum; Ultraviolet light; Polymerase η; Recombination; hMre11; p53

1. Introduction

> The true men of action in our time, those who transform the world, are not the politicians and the statesmen, but the scientists. When I find myself in the company of scientists, I feel like a shabby curate who has strayed by mistake into a drawing room full of dukes (W.H. Auden).

The field of DNA repair began in the 1960s with simple "cut/patch" (or "patch/cut") models for the mechanism of nucleotide excision repair (NER). Contrasting those with what we know now, one begins to appreciate the impact of D. Bootsma and the work he stimulated. Dirk and his colleagues have touched on all aspects of NER, and placed its study firmly within the context of human genetics (Table 1).

Once xeroderma pigmentosum (XP) was identified as a human disease caused by mutations in the NER system [1], Dirk elaborated extensively on its implications. A cursory survey illustrates his many

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Table 1
Representative examples of the contributions of D. Bootsma to the development of understanding of nucleotide excision repair

Xeroderma pigmentosum and ataxia telangiectasia complementation groups	[77–79]
Chromosomal stability and NER	[80]
The molecular defect in the XP variant	[81]
Cloning of NER genes	[82–85]
Functional characteristics of NER genes	[86–88]
DNA repair and circadian rhythms	[89]
Mouse models of NER disorders	[90–92]

contributions to the identification of multiple complementation groups in NER, cloning of many of the genes involved and developing mouse models (Table 1). His contributions and those of his colleagues in the Netherlands, led to a light-hearted description of them at a 1993 Congress in Denmark as the "Dutch Army". Their leadership and impact on the field brings to mind a notable phrase in Sir Richard Attenborough's play "Oh What A Lovely War" (film version 1969). A British officer kneels in prayer before battle imploring, "... Lord, give me victory before the American (Dutch?) marines arrive!"

We have lived through amazing times: from the discovery of the structure of DNA to the sequencing of complete genomes from viruses, prokaryotes, single cell eukaryotes to man. Our understanding of the role of DNA repair has similarly grown in complexity and importance. Interestingly, the possibility (or necessity) of DNA repair was not recognized when DNA was first described structurally [2]. The implications of the double stranded structure, however, in providing an inherent redundancy in genetic information was not lost on the developing field of DNA repair. DNA repair is now incorporated into the catch-all-phase of "genomic stability" and is a major component of all genomes. Most organisms, from the minimal bacterium mycoplasma to multicellular vertebrates contain suites of enzymes for repair or maintenance of genetic stability, and even a few large viruses (e.g. T4 phage) contain a few repair genes. The concept infiltrates our understanding of many of the major macromolecular and organizational processes in biology including genetic variation, evolution, meiosis and mitosis, cell cycle control, differentiation, DNA–protein interactions, transcription, replication, recombination, and telomere maintenance. Clinical importance may also be evident in the association of DNA repair with cancer, stroke, neurodegeneration and aging [3].

Now that the millennium has turned, this is a time to pause, contemplate, and ask questions about why DNA repair takes the form it does, and what discoveries the next generation will make that build upon the foundation that Dirk and his colleagues have laid.

Three general areas of interest arise concerning future studies NER, in particular, and by implication other repair systems:

1. Do we know all possible strategies for NER in all organisms?
2. How is NER integrated and regulated in cells and tissues?
3. Does DNA replication represent a new frontier in the roles of DNA repair?

2. Do we know all possible strategies for NER in all organisms?

Ultraviolet (UV) damage to DNA is one of the best understood, ubiquitous, environmental challenges presented to living organisms, and DNA photochemistry has driven the evolution of DNA repair systems in many organisms [4]. The nucleotide excision replacement (NER) system appears to be a dominant paradigm that has evolved in at least two major kingdoms, the prokaryotic and eukaryotic, to deal specifically with UV-induced photoproducts. Several mechanisms are currently known: two NER mechanisms (prokaryotic and eukaryotic) and several single enzyme systems (pyrimidine dimer glycosylase/AP lyase and UV endonuclease) [5]. The two NER systems include the prokaryotic *UvrABC* system and the eukaryotic *XPA–G* (human), or the homologous Rad3 epistasis group (*S. cerevisiae*). The single enzyme mechanisms are found more commonly in prokaryotes and single cell eukaryotes, and there is little evidence for these in multicellular eukaryotes at present. One is the T4denV type that is a pyrimidine dimer glycosylase/AP lyase resembling the base excision mechanism for alkylation and oxidative damage [6]. The other is a UV endonuclease that cleaves the phosphodiester bond 5′ to a dimer [7,8].

The prokaryotic and eukaryotic NER systems both operate on the same principle in which the recognition functions and catalytic functions are separated

[9]. One set of proteins recognizes damage, and their binding creates a nucleation center onto which the excision system downloads and carries out enzymatic cleavage at sites distant from those of the original damage [5,10].

The prokaryotic NER involves three proteins that carry out the complete process of damage recognition and excision: UvrA, UvrB, and UvrC [5,10]. UvrC is the ultimate nuclease that cuts the damaged strand four nucleotides 3′ from the dimer followed by a cleavage seven nucleotides from the 5′ side [11]. The 12 nucleotide oligonucleotide is released by the combined action of UvrD (helicase II), DNA polymerase I and DNA ligase [5]. The resulting patch is 12–13 nucleotides in length, which corresponds to the footprint of the UvrB:UvrC nuclease on the damaged DNA. This size is characteristic of most prokaryotic NER systems.

The eukaryotic NER system, either that associated with the *S. cerevisiae* Rad 3 epistasis group, or the human *XPA–G* system, operates on the same principle as the prokaryotic NER system, but with very different players. The process involves two branches: transcription coupled repair (TCR) and global genome repair (GGR) [5]. Damage recognition by TCR may occur by the stalling of RNA polymerase II transcription at a damaged site, followed by displacement and downloading of components of the NER system. Damage recognition for GGR involves the XPC/hHR23B complex [12], and the XPE heterodimer (p48/p120) [13]. Both branches, TCR and GGR, converge on the common damage binding complex of XPA/RPA to which the excision nucleases XPG and XPF/ERCC1 bind and carry out the 3′ and 5′ cleavages, respectively [14].

The eukaryotic NER process results in the removal of a 27–29 nt oligonucleotide containing the photoproduct by precisely positioned cleavages 5 nt on the 3′ side of the photoproduct, and 24 nt on the 5′ side [15]. The resulting gap is filled in by the combined actions of DNA polymerase δ or ϵ, proliferating cell nuclear antigen (PCNA), single-strand binding protein (RPA) and ligase [16].

The NER system for repair of UV damage must have been one of the early repair systems to evolve, along with other strategies for survival of living cells in adverse environmental conditions. A recent discussion of the evolution of the three biological kingdoms suggested that there might have been an early period, before the kingdoms differentiated, during which extensive horizontal gene exchange occurred [17]. This would be a period during which the major modalities of DNA repair may have first appeared. This may account for common motifs that are detected across all kingdoms today, especially the helicase motifs [18]. Since two qualitatively different systems, in prokaryotes and eukaryotes, have evolved to achieve the same end, what mechanisms do archea employ? Is archeal NER similar only in overall strategy, with quite different components than prokaryotes or eukaryotes, or is it a minor modification or one of these known mechanisms? Do the archea therefore represent as highly evolved a kingdom as prokaryotes and eukaryotes, or are these organisms closer to the "last universal common ancestor" of all living things [18]? We have attempted to resolve some of these issues by a database search of fully sequenced archeal and other genomes (K. Karplus, J.E. Cleaver, unpublished studies).

A single or few defining genes can be selected for each of the four pathways for UV repair described above, and used for a sequence-based search of fully sequenced archeal genomes. We searched for homologs of the T4denV glycosylase, the SP-UVDE endonuclease, the *UvrA*, *B* & *C* genes and the eukaryotic *XPA/Rad14* genes. In prokaryotes and eukaryotes there are already examples of organisms that function with only one or with several of these systems, so functional redundancy is already evident in even small genomes.

In eukaryotes, the components of the NER system show considerable variability. Whereas XPA, the central damage recognition protein in eukaryotic NER, is present in *S. cerevisiae*, *Drosophila*, *C. elegans* and mammals, homologs to the human XPC and XPE components are more variable. XPC can be identified in *S. cerevisiae*, *C. elegans* and *Drosophila*. XPE, however, could only be clearly identified in *C. elegans* and not at present in *Drosophila* or *S. cerevisiae*, but has been reported in slime mold [19].

Initial searches of archeal genomes using BLAST did not reveal any strong similarities for *T4denV*, *SP-UVDE*, *UvrABC*, and *XPA/Rad14*. We therefore used these genes as seed sequences in more exhaustive searches. Initially, using the SAM-T99 search method described in www.cse.ucse.edu/research/compbio web page that uses a hidden Markov model [20], we found no homologies to *T4denV* in six different

Table 2
Matches detecting similarities for *E.coli* NER genes in *M. jannischii*

Gene	Match	Score[a]	Name/provisional annotation
UvrA	MJ1242	1.3e-110	MeCoMreductase A2
	MJ1662	2.9e-107	MeCoMreductase A2
UvrB	MJ0669	1.4e-129	ATP dependent RNA helicase, e1F-4a
UvrC	MJ1505	2.4e-19	ATP dependent RNA helicase, e1F-4a

[a] The score represents the number of false positives expected by chance.

Table 3
Matches identified for Human NER genes in *M. jannischii*

Gene	Match	Score[a]	Name/provisional annotation
XPA	None		
XPB	MJ1505	2.0e-95	ATP dependent RNA helicase
XPB	MJ0669	2.0e-71	ATP dependent RNA helicase
XPC	None		
XPD	MJ0942	2.9e-102	ATP dependent RNA helicase
XPD	MJ1505	9.2e-08	ATP dependent RNA helicase
XPE	None		
XPF	MJ1505	9.1e-222	ATP dependent RNA helicase
XPF	MJ0669	7.0e-10	ATP dependent RNA helicase
XPG	MJ1444	9.8e-100	DNA repair protein, XPG/Rad2 homolog

[a] The score represents the number of false positives expected by chance. In this search the resulting matches are due to the presence of common helicase motifs and do not necessarily indicate that the genes identified have functions corresponding to the *XP* genes.

archeal genomes nor to the *SP-UVDE*. In contrast, we did find similarities to the *UvrABC* system (Table 2) and to helicase motifs corresponding to the *XPB*, *D*, *F*, *G* genes of the eukaryotic system (Table 3) in the genome of *M. jannischii* and other archea (Table 4). Therefore, it is likely that the archea have a similar NER system to prokaryotes. The helicase motifs persisting throughout all kingdoms may represent a fundamental mechanism of strand discrimination and translocation, and the similarities to the *XP* genes may not represent gene products carrying out the same functions. The sequences that have been identified are only strong for *UvrB* (Tables 2, 4). The *UvrA* sequence homologies may be contaminated by ABC transporter, ATP-binding proteins and the methyl coenzyme M reductase system component A2, making it difficult to tell whether the strong hits are homologs or not. Both "contaminants" come into the alignments even with fairly strict thresholds.

Extending this search to a series of sequenced genomes indicates that it is most likely that the archeal genomes contain an NER system closely similar to

Table 4
Probable (+) and possible (+?) homologs for the *E. coli UvrABC* repair system in archeal species and the bacterium *Mycoplasma genitalium*

Organism	Accession nos.	*UvrA*	*UvrB*	*UvrC*
Aeropyrum pernix	L43967	+?	+	+?
Archaeoglobus fulgidus	AE000782	+?	+	+?
Methanococcus jannaschii	L77117	+?	+	+?
Pyrococcus abyssi	AL096836	+?	+	+?
Pyrococcus horikoshii	AP000001	+?	+	+?
Methanobacterium thermoautotrophicum	AE000666	+	+	+
Mycoplasma genitalium	L43967	+	+	+

that of *E. coli* (Table 4). This would be consistent with previous evidence showing that the halobacteria can excise pyrimidine dimers and [6-4] photoproducts [21] and that *M. thermoautotrophicum* extracts can repair DNA damage with a patch size of 10–11 nucleotides [22]. In one report, however, on the sequence of the *Pyrobaculum aerophilum* genome, no evidence for a *UvrABC* system or UV endonuclease or dimer glycosylase was evident [23]. Biochemical evidence for a SP-UVDE activity in *P. aerophilum* extracts (R.D. Wood, personal communication) suggests that a more extensive search for sequences that will be representative of this class of enzyme in archeal genomes is warranted. There is also the possibility that the archea may be a heterogeneous group with varied composition of their UV repair systems. The NER system of this organism therefore appears to resemble that of *E. coli*, although the small difference indicates subtle differences in the conformation or size of the protein components. Conceivably, the principle of NER may be recognizable in archea, but the individual components may be radically different.

The strong homology between an archeal helicase sequence MJ1444 and the human XPG nuclease suggests that they may have related functions (Table 3). This could be that of strand-specific scanning of the DNA helix, because XPG is required for cleavage of single-strand/double-strand junctions in a strand-specific manner [24]. Since the yeast equivalent, Rad2 is a strong homolog of MJ1444 and is involved in repair and recombination in yeast, the archeal gene function may be more consistent with a recombination function than NER. These surveys suggest that helicase motifs are primitive sequences in proteins that are required to bind and translocate along DNA for a variety of transcription, replication, repair and recombination functions [24,25].

These helicase motifs suggest a scenario for the evolution of the complex DNA repair systems in which helicases are involved in many strand-specific functions, such as transcription and replication. The earliest damage recognition functions may have been carried out by stalled RNA and DNA polymerases. A requirement for specific damage recognition proteins for non-transcribed DNA in eukaryotes (XPA, XPC, XPE), that are apparently absent from prokaryotic and archeal genomes, may be a late evolutionary development. These genes, *XPA, C, E,* would become more important in organisms that segregate their genomes into functionally separate regions of both transcriptionally active and inactive genes, such that repair of the two transcription states can then be regulated differently. The absence of homologs of XPA, XPC and XPE in prokaryotes and archeal genomes may therefore suggest that the coupling of repair to transcription is an early event in the evolution of repair systems.

3. How is NER integrated and regulated in cells and tissues?

Prokaryotes and eukaryotes regulate NER in quite different ways, and understanding transient and persistent alterations in NER in response to damage presents a major challenge. In prokaryotes, the components of NER are present at very low levels until induced by de novo synthesis in response to DNA damage [5]. Eukaryotes, especially multicellular organisms, respond instead to DNA damage by altering the activity rather than the expression of many DNA repair enzymes. The inducible response of prokaryotes seems focused on the survival of the individual cells, whereas multicellular eukaryotic organisms substitute an apoptotic response that preferentially eliminates damaged cells [26]. DNA damage to mammalian cells initiates transient phosphorylation cascades in which many different proteins and many sites on individual proteins undergo phosphorylation and dephosphorylation by a variety of kinases and phosphatases, including ATM, ATR, Chk2, p21, DNA-PK, casein kinase and others [27,28]. The substrates and kinases are still less well understood for UV damage as compared to ionizing radiation [29], but the long-standing sensitivity of UV damaged cells to caffeine may involve inhibition of these kinases [30]. Not all phosphorylations are functional, however. A very large number of sites can be phosphorylated on p53, but elimination of these sites by mutagenesis seems to have no functional consequences [31]. Similarly, phosphorylation of the p34 component of RPA, an essential component of NER, occurs in response to UV irradiation, but is not required for the protein to function in NER but may be involved in cessation of DNA replication [32,33]. The functional significance of many of these phosphorylations is, therefore, still a matter of conjecture. They may represent, for example, a transient,

widespread "alarm" response, from which only a few alterations are actually employed for the particular damage on hand.

The discovery of multiple complementation groups by Dirk Bootsma and his colleagues (Table 1) made the field appreciate how many gene products are required for mammalian cells to remove photoproducts from DNA. For a large part of the early development of NER, during which Dirk and his colleagues identified many of the components, genes, and made animal models, it was possible to view NER in isolation. NER appeared to function as a discrete excision system insulated from the rest of the cellular regulatory networks. An emerging challenge is to understand how NER and other repair systems are regulated in dividing and differentiated cells, and in normal and malignant transformed cells. The discovery that several components of NER were also components of the transcription factor TFIIH was the first recognition that NER was not an isolated system [34]. Discovery that the TCR coupling factors, CSA and CSB, were involved in ubiquitination of RNA pol II [35], and that TCR also required the function of mismatch repair [36], were further indications that understanding the integration of NER into cellular regulatory pathways was important. Of emerging interest is the role of p53 in regulating NER [37], and examples of down-regulation of NER in certain normal and tumor tissues.

The first linkage of p53 to NER was the identification of binding sites for XPB and RPA on p53 [37]. This implied that p53 could play a role in regulating TCR. Further evidence for this came from studies in Li-Fraumeni syndrome cells that carry mutations in p53, and in transformed cells [38–41]. Recently, evidence has been presented that p53 regulates both TCR and GGR, but the retinoblastoma gene product, Rb, regulates only GGR [42]. One question about these studies is that the evidence of alterations in NER resulting from inactivation of these cellular pathways mainly comes from high-resolution assays of a small number of specific genes. Comparable studies using total cell assays for pyrimidine dimer excision and unscheduled DNA synthesis did not detect any changes in NER associated with inactivation of p53 and Rb pathways [43], raising questions about the overall significance of these sets of data. This is an important area to investigate further, because the frequent mutation of p53 in human cancers [37] suggests that most tumors should have some derangement of NER. The derangement may play a role in the progression of tumors or present an opportunity for therapeutic intervention. Two examples of this in human tissue and tumors illustrate these possibilities.

Testicular tissue and tumors are defective in expression of XPA, which helps explain the sensitivity of these tumors to cisplatin chemotherapy [44]. The mechanism of down-regulation is still not explained. This is not the only evidence for repair deficiency in the testis, however. Ligase III, which is involved in base excision repair (BER) through its interaction with XRCC1, undergoes alternative splicing in the testis, eliminating its XRCC1-interacting exon [45]. We have also recently found that the major damage-specific polymerase corresponding to the XP variant group, pol η, undergoes alternative splicing in the testis, and to a lesser extent in some other tissues, so as to eliminate the exon containing the ATG start codon (M. Wernick, J.E. Cleaver, unpublished observations). The testis is therefore effectively a triple mutant, lacking significant functions in all the main pathways for error-free processing of DNA damage. We have found that loss of these pathways, especially XPA and pol η, results in recruitment of the hMre11/hRad50/Nbs recombination system, suggesting that down-regulation has a specific function in enhancing recombination during male meiosis.

Malignant melanoma is another example of potential DNA repair linkages. The role of UV exposure in inducing melanoma is still a matter of great controversy [46]. Recent molecular analysis of malignant melanoma [47] allows an alternative interpretation. A primary event in malignant melanoma appears to be loss of the region of chromosome 9p carrying the *p16* familial melanoma gene that is part of the cell cycle checkpoint pathway [47]. Inspection of the chromosomal losses and gains reported in these melanomas indicate that subsequent to *p16* losses, there are additional losses of 9q including the basal cell nevus and *XPA* genes, amplification of 6p carrying the pol η gene (*hRad30A*), and loss of 6q containing the pol ζ active subunit *hRev3*. These multiple disturbances in genes that play an important role in the processing of UVB damage could play a complex and variable role in the UVB response of melanocytic cells and early tumors. The complexity and confusion in the role of UV exposure in melanoma induction could therefore

be explained in part by dysregulation of several repair pathways subsequent to the initiation of malignancy.

DNA repair has therefore many interesting and important avenues remaining for exploration in normal and malignant tissue and its prospect as a major target and tool in human cancer is highly promising.

4. DNA replication: a new frontier for DNA repair?

Somewhat prematurely, I linked NER with the response of DNA replication to UV damage [48], on the basis of reduced postreplication repair in NER-defective XP cells [49] and the failure of DNA replication to recover in Cockayne syndrome cells [50]. But the technical tools for pursuing this connection were not yet available, and this notion was soon eclipsed by the demonstration of a linkage between transcription and repair, a discovery strongly associated with the research stimulated by both Dirk Bootsma and Philip Hanawalt [34]. Only in the past few years has pursuit of the connection between DNA repair and replication resurfaced with a flurry of exciting new discoveries.

The eventual solution of the human disease XP as an NER-defective disease was hampered for a long time by the puzzle that a significant number of patients with diagnosed XP lacked NER deficiencies [51]. These, the XP variants, were finally solved in 1999 [52–55]. The solution represented a dramatic linkage between the repair and replication of UV-damaged DNA in generating essentially identical clinical symptoms [56]. The identification of the *XP* variant gene product as the error-prone polymerase η initiated a remarkable flood of discoveries of multiple low-fidelity polymerases, of which at least nine have been listed to date (Table 5) [57]. These polymerases act individually, or in concert, to complete replication of damaged DNA, and differ in their specificity for different DNA substrates. Some can insert bases opposite non-coding lesions, whereas others extend DNA from a misincorporated base [58]. In contrast to the replicative polymerases that generally have a high fidelity of 10^{-5}–10^{-7}, most of these damage-specific polymerases have a low fidelity of about 10^{-2} (Table 5). Their properties raise many interesting issues, not least of which are: (a) how do low-fidelity polymerases contribute to error-free replication of UV damage? (b) how is repair, replication and recombination coordinated at the replication fork? and (c) what is the mechanism underlying the association between many of these low-fidelity polymerases and sister chromatid cohesion?

4.1. Low-fidelity polymerases and error-free DNA replication

Polymerase η, that is associated with the XP variant, reduces UV-induced mutation rates in vivo, when under endogenous regulation. But in vitro it has an

Table 5
Damage-specific, error-prone, DNA polymerases in eukaryotic cells[a]

Pol	Alternate name	Chromosome	Reported functions[b]
η	*hRad30A*	6p21	Error-free bypass of cys–syn dimers (not [6-4] or AAF)
ζ	*hRev3/7*	6q22	Mutagenic extension of pol ι mispaired bases, & abasic sites, high-fidelity polymerase, hMAD2 interaction (*hRev7*), essential for viability
θ		3	Crosslink repair?
ι	*hRad30B*	18q21	Incorporation opposite highly distorting lesions ([6-4], abasic), extended by pol ζ, *hRad30A* homolog
κ	*hDinB1*	5	Sister chromatid cohesion; bypass of AAF & abasic sites (not UV photoproducts, or CisPt), essential for viability in yeast
λ		10	
μ		17p13	Lymphoid tissue
Eso1p			Sister chromatid cohesion, homology to pol η
hRev1			dCMP terminal transferase, UV mutagenesis

[a] Additional unpublished information on pol λ, pol θ, and pol μ from L. Loeb, University of Washington. Chromosome locations for *hRad30A* and *hRev3* confirmed by FISH (J.E. Cleaver, M. Wernick, unpublished experiments).

[b] References for pol η [53–55], pol ζ [58,73], pol ι [58], pol κ [74], Eso1p [67], and *hRev1* [75].

error rate of the order of 1% even on undamaged DNA [53,55]. We have recently found that high levels of expression of the *hRad30* gene on episomal vectors from heterologous promoters can be toxic to cells (M. Thakur, J.E. Cleaver, unpublished observations); only low level expression from chromosomally integrated genes results in complementation [59]. In our own experiments, for example, high levels of expression of *hRad30A* from episomal vectors proved toxic to cells. But once the same vectors were linearized, and integrated into the chromosome at lower copy numbers, the expression of *hRad30A* complemented the XP variant phenotype (M. Thakur, J.E. Cleaver, unpublished experiments). These experiments suggest that in vivo there is a highly regulated system for channeling the functions of pol η and quenching its rampant pro-mutagenic activity.

One possible mechanism of regulation in vivo is that of restricted access to the appropriate substrates. The polymerase may only be expressed, or only find access to a DNA template, under conditions of UV-induced replication arrest. Arrested replication forks have extended single-stranded regions that would be more accessible to these supplementary polymerases [60]. Access might be regulated further through binding to regulatory proteins, intracellular sequestration, and/or posttranslational modification.

Another possibility comes to mind, that I think of as one of "Nature's Jokes". The low-fidelity polymerases have a range of substrate preferences (Table 5), including UV photoproducts, large DNA adducts, abasic sites, and as will be suggested below, a number of atypical or non-canonical DNA structures. That a pathway for processing UV damage involving pol η is error-free may be fortuitous and unique for UV. The major UV-induced photoproducts involve thymines and cytosines; polymerase η, following the "A" rule, has a preference for inserting purines, first A and then G. Inserting purines opposite UV-damaged pyrimidines will result in accurate copying of the DNA template. An unusual case in which two wrongs can make a right!

4.2. Coordination of repair, replication and recombination at the DNA replication fork

Early studies of DNA replication in human cells showed that bromodeoxyuridine (BrdUrd)-labeling highlighted concentrated, clustered foci in cell nuclei that were regions of DNA replication. It was not clear at the time why these clusters were so large and what other factors they contained. As the result of recent work, it is likely that these foci are in fact fidelity factories in which many functions of DNA repair and recombination cooperate in ensuring faithful replication of the whole chromosome structure [61].

We recently found that one of the functions of these replication foci involves channeling the processing of a blocked replication fork into either a bypass mode or a recombination mode (Fig. 1). Channeling into a recombination pathway was enhanced in cells lacking p53 as a result of either SV40 [43,62] or HPV16(E6/E7) transformation (Limoli and Cleaver, unpublished observations). Recombination involved

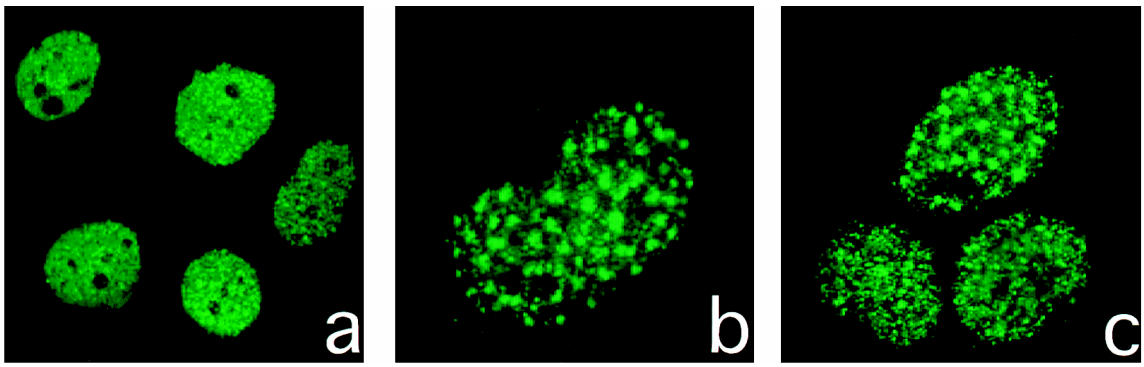

Fig. 1. Immunofluorescence of hMre11 protein, 4 hr after UV irradiation with 13 J m^{-2} UVC light on human SV40 transformed XP variant (XP30RO) cells; (a) control; (b) and (c) UV irradiated cells showing hMrel11 foci induced by UV damage.

Table 6
Involvement of hMre11 recombination in UV damaged cells lacking NER or replicative polymerases[a]

Genotype	Foci positive cells (%)
Normal	0.5
NER-defective (*XPA*)	3
Pol η-defective (*XPV*)	14
Pol ζ-defective (*hRev* 3)[b]	4.5
Pol η, pol ζ, double mutant[b]	23.6

[a] Recombination involving hMre11 foci occurs at DNA replication forks following UV irradiation and are coincident with PCNA [62]. Foci are reported for SV40 transformed cells; recombination is suppressed in cells with normal p53 responses [62].

[b] Cells lacking pol ζ were developed by transfection of an episomal vector expressing a ribozyme that targeted exon 4 of *hRev3* [76]. Loss of *hRev3* expression was confirmed in normal and XP variant cells by RT-PCR.

the hMre11/hRad50/Nbs complex, and was enhanced in cells that were defective in NER, pol η, pol ζ, or p53 (Table 6, Fig. 2). Our results indicate that loss of either pol η or pol ζ resulted in increased frequencies of cells positive for hMre11 foci, and that the frequency in the double mutant deficient in both polymerases was additive. This suggests that pol η and pol ζ may operate on parallel pathways (e.g. for cyclobutane dimers and for [6-4] photoproducts) independent of each other.

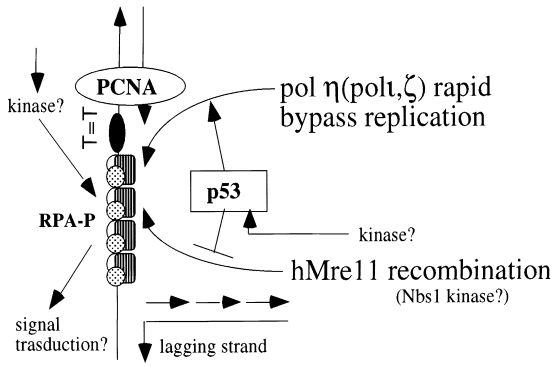

Fig. 2. Possible structure of an arrested leading strand of a DNA replication fork. The extended single strand region is coated by RPA that becomes phosphorylated and acts to initiate signal transduction and attract components of the replication fork recovery mechanisms. The most rapid recruitment is for pol η (hRad30A) that replicates pyrimidine dimers accurately; subsequent recruitment of other low-fidelity polymerases for mutagenic bypass of [6-4] photoproducts and abasic sites then occurs. p53 enhances replicative bypass and suppresses recombination; in its absence (e.g. SV40 large T) the hMre11/hRad50/Nbs1 recombination complex is recruited in at least half of the S phase cells.

The substrate specificities of several of the polymerases (Table 5), together with the action of p53, may establish a hierarchy for the response of human cells to UV damage, that depends on the particular genetic constitution of the irradiated cells. The first, preferred, response in normal cells is accurate replication of UV damage by pol η [56]. A second, slower response, that is enhanced in pol η-defective cells, involves mutagenic bypass and chain extension through the actions of pol ι and pol ζ (Table 5) [58,63]. This step in pol η negative cells may involve kinase activation in vivo, since kinases and replication bypass are both inhibited by caffeine [30,63]. If replicative bypass cannot occur, and cells cannot mount a normal p53 response, then cells recruit the hMre11/hRad50/Nbs1 recombination machinery to alleviate a replication arrest [62]. In NER-defective cells, also, these processes are all enhanced due to the persistence of high levels of unexcised damage presented to DNA replication forks (Table 6) [62,64].

We thus envisage replication foci as regions containing a large number of repair and recombination factors, coordinated by mutual interactions and p53 activity. Elucidation of the multiple functions and their hierarchy of importance will clearly be a new frontier for much interesting research.

4.3. The association between low-fidelity DNA polymerases and sister chromatid cohesion

Although the low-fidelity polymerases have been detected first by their role in replication of damaged DNA and mutagenesis, several have been shown to have major roles in sister chromatid cohesion [64]. The non-catalytic hRev7 component of pol ζ interacts with a mitosis-associated protein, hMAD2 [66]. Polymerase κ [65] and a pol η homolog (Eso1p) [67] were also shown to play a role in the cohesion of sister chromatids. We also showed that the loss of pol η in p53 negative cells resulted in greatly elevated levels of sister chromatid exchange (SCE) [43]. This represented indirect evidence that resolution of chromatids during replication and mitosis was involved with the function of pol η.

One possible explanation of the linkage between sister chromatids and the low-fidelity polymerases

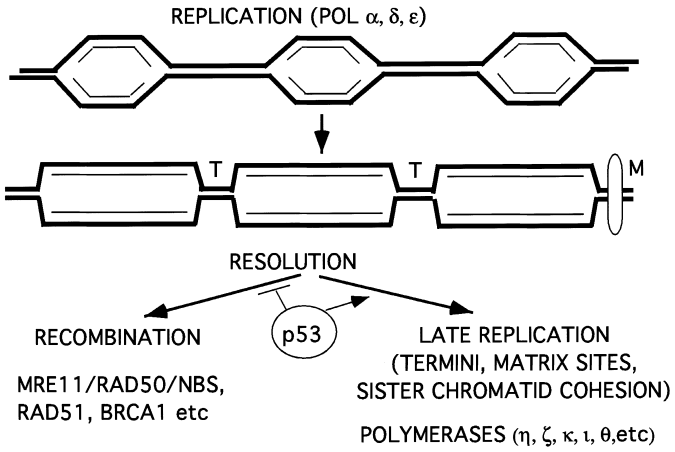

Fig. 3. Bidirectional DNA replication using processive polymerases, pol α, δ, ε, from multiple origins in human cells results in a need to resolve complex highly coiled structures at replication termini (T) and at attachment points to matrix proteins (M). Resolution can occur through replication involving low-fidelity distributive polymerases (pol η, ζ, κ, ι, θ, etc.) and recombination mechanisms involving protein complexes containing hMre11/hRad50/Nbs1 or Rad51/Brca1. Resolution of these structures is required before sister chromatid separation and can result in exchanges visualized as SCEs. The absence of one or more of these polymerases and p53 redirects resolution of blocked forks into a recombination pathway.

(Table 5) is that completion of DNA replication involves resolution of many complex structures at replication termini, matrix attachment points, and other anomalous DNA conformations. Some of these may involve replication of small regions of DNA that can occur extremely late in S or even in G2, and would require the substrate flexibility represented by the low-fidelity polymerases (Fig. 3). This notion would help explain why some of the cohesion-associated polymerases appear essential for viability (pol κ, pol ζ), whereas others (pol η) appear more devoted to damage bypass, and inactivating mutations are tolerated.

An emerging paradigm therefore suggests that we should perhaps, exercise a broader view of the functions, interactions and regulation of NER repair enzymes. Several NER genes have been found to be multifunctional involving other repair systems, e.g. *XPG-nth*, an association between the NER 3′ nuclease with the glycosylase for excision of oxidative damage [68] as well being part of fundamental transcription processes (*XPB*, *D*). Losses in the function of some repair enzymes can result in the recruitment of others (e.g. *XPA*, *XPV* deficiencies increase the recruitment of hMre11 recombination complexes during DNA replication [62]).

A traditional view has been to regard NER, BER, etc. as discrete and separate processes. Recently, however, several were found to be members of replication complexes (uracil-*N*-glycosylase, 5-MeC transferase, PARP, XRCC1) [69–72]. A newer view could regard familiar processes such as NER as temporary associations made from a larger pool of DNA-interacting proteins. These particular complexes would associate according to the specific challenges set by DNA damage or unusual DNA conformations. A recent example of such a large complex was termed the BRCA-complex (BASC) [61] that contained many known polymerases, repair enzymes, and recombination proteins. This probably represents one class of many possible complexes. Since the BASC structure was formed in HeLa cells lacking p53 [61], that is known to be associated with many of the components of this complex [37], it is likely that the BASC is only one of many possible transient complexes. Complexes with different members are likely to be found in damaged as compared to undamaged cells, and in p53 positive and negative cells.

This field of DNA repair and replication illustrates what has been endemic in the field that Dirk and his colleagues have so eminently pioneered. There is always another surprise around the corner. We envy the

next generation the excitement of the investigation, the tools available, and the sense of progress and achievement that lies ahead.

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