COMMENTARY

Pathways of human cell post-replication repair

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Mutagenesis, clastogenesis, and carcinogenesis, may all be S-phase dependent processes within carcinogen-damaged human cells. Carcinogens have been shown to inhibit replicative DNA synthesis in S phase cells and the mechanisms of inhibition have been identified. It is proposed that the sequelae of carcinogen action (mutations, sister-chromatid exchanges, chromosome aberrations) are the consequence of the production of lesions in the DNA template which interfere with the ability of DNA polymerase to synthesize a complementary strand without error. Mis-instructive lesions in the template give rise to base-substitution mutations in nascent strands as DNA polymerase inserts an incorrect but complementary base. Non-instructive base lesions and sterically interfering bulky adducts in the template inhibit DNA polymerase and cause the growing points of nascent DNA strands to be blocked. This blockage perpetuates discontinuities in daughter strands. These discontinuities are eliminated by a process known as post-replication repair. Blocked growing points may be relieved by un-directed insertion of DNA precursors to span the non-instructive lesions. Transient dislocation of the primer terminus from the damaged template may occur at palindromic or repetitive sequences. Reannealing of the primer terminus beyond the site of damage may allow bypass of blocking lesions with a consequence of deletion or insertion of genetic information. DNA at the site of blocked growing points may be a substrate for other enzymes involved in DNA metabolism. Single-strand gaps in daughter strands may be recognized by RecA-like proteins which catalyze paranemic invasion of sister duplex strands. Recombination intermediates generated at sites of blocked growing points may be resolved by a pathway that produces either sister-chromatid exchanges or the insertion of a patch of parental template DNA within the daughter strand. Single-strand-specific endonuclease may attack regions of denatured DNA at blocked growing points producing double-strand breaks which appear to be intermediates in the formation of chromatid aberrations. The utilization of each of these pathways of post-replication repair will depend upon the precise structure of the template lesion, the sequence context in which the lesion is embedded in the template strand, and stochastic processes.

Introduction
Numerous studies have revealed the specific vulnerability of S phase cells to carcinogenesis. For rat hepatocytes in vivo the initiation of carcinogenesis by a subthreshold dose of chemical carcinogen and the induction of cancer directly by a suprathermal dose both occurred with greatest yield when proliferating target cells were early in the S phase of the cell cycle (1-3). Proliferating hepatocytes in early G1 were significantly less sensitive. Similarly, in in vitro model systems using diploid human fibroblasts, transformation occurred with measurable frequency only when synchronized cells were treated with chemical carcinogen or 254 nm ultraviolet (UV*) radiation just before or during the S phase (4,5). Treatment in early G1 appeared to be without effect (4,5). Mutation at the hypoxanthine guanine phosphoribosyl transferase locus displayed a similar S-phase dependence (6-8). Moreover, the formation of chromatid-type chromosomal aberrations in chemically damaged human lymphocytes also was an S-phase dependent process (9). Seemingly, during the replication of damaged DNA, genetic alterations occur which can change the structure or activity of critical gene products (Figure 1). The lower sensitivity of cells in early G1 may be reasonably ascribed to the operation of error-free pathways of DNA repair prior to DNA replication (4,6,7).

Chromosome-type chromosomal aberrations are S-phase independent genetic alterations (10) which may be produced by damage incurred during the pre-replicative G1 phase of the cell cycle although they are generally identified during mitosis. Pre-mitotic DNA repair also appears to reduce the frequencies of these lesions (10). Because the chromosome-type chromosomal aberrations do not result from the replication of damaged DNA, they will not be considered further here. The reader is referred

*Abbreviations: UV, ultraviolet radiation; AP sites, apurinic/apyrimidinic sites; BPDE, (±)-7,8-dihydroxy-9,10-epoxy-9,10-ethylenedioxy-1,2-benz[a]pyrene; XP, xeroderma pigmentosum; AAAF, N-acetoxy-2-acetylaminofluorene.
A summary of the current understanding of DNA replication previous reviews (23-25) to establish historical context. post-replication repair, which is the subject of this work. to provide additional perspective on the phenomenon of in carcinogen-damaged human cells will be presented below been applied to describing the effects of carcinogens on DNA Perpetuation of discontinuities in nascent DNA and is beyond the scope of this work. The reader may refer to several a crucial step in carcinogenesis (20—22), and much effort has It is not a new idea that replication of damaged DNA may be hypothesized that at least some of these critical alterations occur when multi-enzyme DNA replication complexes (16,17) orientation in the template (D). Reaction of BPDE with the N2 of guanine adds a large bulky adduct (E). Each of the template lesions depicted in C, D and E appear to block DNA polymerase and perpetuate discontinuities in nascent strands (i.e. free 3' OH primer termini). to reviews of the phenomenology of induction of chromosome aberrations (11,12).

The spectra of carcinogen-induced alterations in the ras gene family (13—15) support the view that misreplication of damaged DNA may be a critical event in carcinogenesis. However, the precise natures of the genetic alterations necessary to produce a malignant neoplasm remain to be identified. It is our working hypothesis that at least some of these critical alterations occur when multi-enzyme DNA replication complexes (16,17) encounter carcinogen-induced lesions in the DNA template. An understanding of the effects of carcinogen-induced damage on DNA replication may provide insight to the mechanisms of activation of oncogenes (18,19).

Perpetuation of discontinuities in nascent DNA and a definition of post-replication repair

It is not a new idea that replication of damaged DNA may be a crucial step in carcinogenesis (20—22), and much effort has been applied to describing the effects of carcinogens on DNA replication. A thorough review of the literature on this subject is beyond the scope of this work. The reader may refer to several previous reviews (23—25) to establish historical context. A summary of the current understanding of DNA replication in carcinogen-damaged human cells will be presented below to provide additional perspective on the phenomenon of post-replication repair, which is the subject of this work.

Post-replication repair may be defined as the process whereby discontinuities, that are perpetuated in nascent daughter strands by the interference of carcinogen-induced template lesions with DNA chain elongation, are eliminated to form continuous daughter strands. Discontinuities are inferred from the synthesis of abnormally small nascent strands in damaged cells, and the elimination of these discontinuities is inferred by the observation that nascent strands eventually grow to larger sizes. The growth of abnormally small nascent strands to larger mol. wts is the biochemical measure of post-replication repair (26—28). It might be presumed that the process of elimination of discontinuities carries certain risk of error and that post-replication repair is, in part, responsible for the formation of genetic alterations in damaged S-phase cells.

Genetic alterations of the base-substitution type may also occur when DNA polymerase encounters damaged bases with altered hydrogen-bonding specificities such as O6-methylguanine and O4-ethylthymine (29,30). These mis-instructive lesions in template strands appear, respectively, as adenine and cytosine to DNA polymerase leading to transition mutations. Mis-instructive lesions that are capable of directing polymerization should not perpetuate discontinuities in nascent strands, and, so, should not require post-replication repair.

There are at least two types of damage to template strands that do perpetuate discontinuities in DNA replication intermediates and for which cells require post-replication repair to produce continuous strands (Figure 2). One type produces a non-instructive lesion that cannot direct DNA polymerase in the addition of the incoming deoxyribonucleotidyl precursor to the primer terminus. This occurrence might be anticipated when polymerase encounters in the template strand apurinic/apyrimidinic (AP) sites. These lesions may be formed under a variety of conditions such as heat- and enzyme-catalyzed depurination of 7-alkylguanine and 3-alkyladenine in carcinogen-damaged cells (31,32) or after enzymatic attack on the glycosyl bond of deoxyuridine residues in DNA by uracil N-glycosylase (33). Uracil residues in DNA may result from deamination of cytosine or from incorporation of deoxyuridine triphosphate (34). Because no hydrogen-bonding information is available at AP sites, these lesions can be expected to produce a halt to chain elongation by DNA polymerase (35).

Another example of a non-instructive template lesion is the UV radiation-induced a-hThy(6-4)Pyo lesion (Figure 2). In this pyrimidine dimer the carbon-4 in the 3' cytosine ring is linked to carbon-6 of the 5'-adjacent thymine ring (36). The amino group at carbon-4 in the 3' cytosine of the dimer is transferred to carbon-5 in the 5' thymine ring. This is due to the instability of the four-membered azetidine ring, which had been formed initially producing a Cy4(6—6; N5)Pyo intermediate (36). The structures of the TC and CC 6-4 dimers (6-4 Pyo) appear to preclude the direction of the incoming deoxyguanosine triphosphate precursor due to the shifting of the cytosine base from its normal orientation and the transfer of the hydrogen-bonding amino group to carbon-5 in the adjacent thymine/cytosine. The destruction of hydrogen-bonding information as well as the altered orientation of the pyrimidine base in the template strand should be non-instructive and so is expected to produce a halt to polymerization.

A second mechanism of inhibiting DNA polymerase is by steric interference. The N-2 guanosyl adduct produced upon reaction of (±)-7r,8-tetrahydrobenzo[a]pyrene (BPDE) with DNA (37,38) might be expected to impede polymerase by its large size (Figure 2).
Post-replication repair

Fig. 3. Structure of a damaged replicon. Blockage of chain elongation and fork progression on the leading template strand and blockage of chain elongation on the lagging template strand may produce a replication intermediate with altered structure. A consequence of such blockage is that regions of single-stranded, nucleosome-free template may be exposed for abnormally long intervals. Protein components of DNA replication complexes are not shown for their interaction with DNA at replication forks has not been resolved. It is likely that regions of the single-stranded template DNA are associated with various proteins such as ssDNA binding proteins and other replication-associated enzymes.

Similarly, the C-8 guanosyl-acetylaminofluorene adduct, which can cause the guanine moiety to flip out from between stacked bases in the template (39), may be sterically interfering or non-instructive depending upon its orientation within the DNA template. The mis-instructive precursor, $O^6$-methyldeoxyguanosine triphosphate appears to impede DNA polymerase by interfering with proper stacking of bases when incorporated into the nascent daughter strand (40). An extreme form of steric interference may be the DNA interstrand crosslink which appears to represent a very effective block to DNA replication in human cells (41) presumably by preventing separation of single-stranded DNA templates.

When non-instructive or sterically interfering lesions are encountered in template strands, they can be expected to block further elongation at the growing points of nascent strands (Figure 2). The necessity for discontinuous synthesis of one of the template strands (42) implies that the presence of blocking lesions on the discontinuously copied template may lead to interruption of the completion of an Okazaki fragment without impeding the continued progression of the replication fork into the parental duplex (43). On the other hand, the presence of such a blocking lesion on the leading template strand may stop continuous synthesis of the nascent strand. This blockage of the continuously synthesized strand has been hypothesized to block further invasion of the replication fork into the parental duplex (28,43,44) leading to cessation of DNA synthesis.

Accumulation of altered structures in damaged replicons

Blockage of elongation of the leading and lagging nascent strands by steric hindrance or non-instructive distortion of template can be expected to produce structures depicted in Figure 3. It may be readily appreciated that the structure of nascent DNA in the region of such lesions is considerably altered. Due to the impedence of fork progression by lesions on the template for the leading daughter strand, regions of the lagging template strand may be forced to exist in a denatured state free of the normal nucleosomal chromatin structure (45). Given the average size of Okazaki fragments of $\sim 100-200$ nt, an equivalent stretch of template DNA may be exposed before a primer site is recognized and retrograde polymerization initiated. As already mentioned, the presence of blocking lesions on the lagging template strand may interfere only with the completion of an Okazaki fragment without affecting fork progression. As a consequence gaps are formed in nascent duplexes as discontinuities in nascent strands are perpetuated. It may be useful here to emphasize that discontinuities in nascent strands are perpetuated by the blocking lesions rather than actually being produced. This is a natural consequence of the discontinuous mode of replication of the lagging daughter strands and the simultaneous synthesis of adjacent regions of chromatin within replicon clusters. Because most S phase cells will contain daughter duplexes at various stages of completion, carcinogens may interact with daughter strands as well as with parental template strands. DNA excision repair in these daughter strands will produce true discontinuities transiently. However, because the frequency of these discontinuities ($5$ breaks in $10^{10}$ daltons (46)) is much lower than the frequency of replicons ($250$ per $10^{10}$ daltons) and the lifetime of repairing sites is short ($1-10$ min (47)), these intermediates in DNA excision repair need not be considered as direct participants in post-replication repair. The operation of DNA excision repair pathways on unreplicated damaged DNA will, however, remove blocking lesions before they are encountered by growing points. Thus, DNA repair will have an indirect effect on post-replication repair by reducing the frequency of formation of blocked growing points. DNA repair
may also play a role in regulating the initiation of replicons as will be discussed further. The operation of post-replication repair pathways on the structures of replicating DNA depicted in Figure 3 eliminates the discontinuities in the nascent daughter strands. Before examining potential pathways of post-replication repair that can eliminate these discontinuities in nascent strands it is necessary to review recent work that has identified the mechanisms whereby S-phase dependent carcinogens such as UV and BPDE inhibit DNA replication in human fibroblasts.

**Mechanisms of inhibition of DNA replication**

Two mechanisms of inhibition of DNA replication have been identified in S phase cells and these are down-regulation of replicon initiation and inhibition of chain elongation by interference with DNA polymerase as described above (28, 48—50). The effect on replicon initiation was observed after treating cells with low doses of UV or BPDE that do not produce measurable loss of cell colony-forming ability (6—8). This down-regulation of replicon initiation in damaged S phase cells should interact with DNA excision repair processes to reduce the probability of genetic alterations. By reducing the number of DNA growing points operating in damaged cells, DNA excision repair has more time to operate on damaged DNA before it is replicated. Thus, the effect on initiation appears to be adaptive and reparative in character and reduces the probability of perpetuation of discontinuities in daughter strands that must be dealt with by post-replication repair. The failure of DNA excision repair pathways to operate ahead of replication forks will enhance the formation of discontinuities in nascent strands (26,48,51) producing an image of defective post-replication repair (27,52). After a low, sub-lethal dose of carcinogen, the inhibition of replicon initiation is maximal 30—60 min after damage is incurred and recovery in the rate of initiation is evident by 120—180 min after treatment (48,49). After higher doses of carcinogen, the effect on initiation can be identified although under most conditions of analysis the effect is masked by the second effect of carcinogens, the inhibition of DNA chain elongation in operating replicons.

Recent work in our laboratory has identified a possible regulator of replicon initiation. When human cells were briefly incubated with low concentrations of inhibitors of DNA topoisomerase II (camptothecins and etoposide (53)) a rapid and long-lasting inhibition of replicon initiation was produced (Figure 4) (W.K.Kaufmann and J.C.Boyer, in preparation). Topoisomerase II has been implicated as an important replicative enzyme in proliferating cells (53—57) and appears to be responsible for separating interwound daughter duplexes. Topoisomerase II mutants in yeast accumulate in G2 (58,59) suggesting that the final stages of chromatid segregation are blocked. Human leukemia cells that have been treated with etoposide to inhibit topoisomerase II also accumulate in G2 after prolonged passage through S phase (60). Recent studies indicate that certain chromosomal changes previously attributed to early M (i.e. early prophase condensation of daughter chromatids) may actually begin in S phase soon after synthesis of the daughter chromatids (61). This suggests that chromatid separation may begin soon after the completion of DNA replication within a replicon cluster, rather than be limited to the few hours of G2. With this model in mind, it is not so surprising that inhibition or interruption of topoisomerase II activity would produce an inhibition of replicon initiation. Newly synthesized daughter chromatids may vacate DNA replication complexes only after topoisomerase II has successfully separated the topologically interwound molecules within the replicon cluster. Initiation of DNA replication in the subsequent clusters of replicons cannot occur until the replication complex is vacated by the newly segregated daughter chromatids. Carcinogens such as UV and benzo[a]pyrene are known to induce the formation of protein—DNA crosslinks (62,63) consistent with the transient formation of the topoisomerase—DNA complex. Distortion of DNA structure in the vicinity of lesions may be sufficient stimulus to induce this reaction and interrupt the normal operation of topoisomerase II. DNA excision repair pathways, while removing the distorting lesions, also relieve the effect of these lesions on topoisomerase II activity and, so, allow resumption of normal replicon initiation. While further work is needed to determine the validity of these speculative ideas, the importance of DNA topoisomerase activities in human DNA metabolism cannot be discounted.

Fig. 4. Inhibition of replicon initiation by etoposide. Log-phase cultures of diploid human fibroblasts were incubated for 10 min with 3 or 30 μM etoposide and then allowed 30 min recovery in drug-free medium (top panel) or were incubated for 10 min with 10 μM etoposide and allowed 30 or 90 min recovery in drug-free medium (bottom panel). Following these treatments cells were incubated for 15 min with 3H-thymidine and then nascent replication intermediates separated by velocity sedimentation in alkaline sucrose gradients. Normalized 3H-c.p.m. represent acid-insoluble radioactivity normalized to a constant cell number (48). Sedimentation was from right to left. The arrows show the position of sedimentation of molecules of half the average replicon size (2 x 10^7 daltons). The initial selective reduction of incorporation of precursor into replication intermediates of half the average replicon size which then spreads with time to include larger intermediates (in fractions 8—14) is characteristic of an inhibition of replicon initiation (48—50).
As the dose of carcinogen is titrated upwards from the low doses that affect replicon initiation only, two additional alterations of DNA replication can be observed which appear to be linked. These are (i) a dose-dependent inhibition of DNA synthesis in operating replicons, and (ii) the production of abnormally small nascent DNA (48,49). The inhibition of DNA synthesis in operating replicons is thought to reflect the interference by template lesions with DNA polymerase leading to cessation of chain elongation at DNA growing points. The production of abnormally small nascent strands is thought to reflect the formation of gaps in daughter strands due to the inability to complete Okazaki fragments (43) and join adjacent replicons. It is also conceivable that some of the small strands simply represent replication intermediates in the reduced fraction of newly initiated replicons whose growth to larger size is slowed by leading strand lesions. As proposed above, blocking lesions in the template DNA inhibit chain elongation and fork progression when encountered by the leading nascent strand but perpetuate the existence of gaps in daughter DNA when encountered by the discontinuously synthesized, lagging daughter strand. Because an average of fifty percent of lesions will be initially formed on the leading strand, when in sufficient density per replica, they can produce a virtually complete inhibition of DNA synthesis in operating replicons (48,49) through blockage of progression of replication forks. For example, a dose of BPDE that was calibrated to produce about 2 adducts per replicon also produced >90% inhibition of DNA synthesis within replicons within 45 min after the treatment (49). The analysis of replication in BPDE-damaged human fibroblasts suggested that each BPDE-guanosine adduct when encountered in template strands impeded DNA synthesis and blocked the elongation of nascent DNA (28).

After doses of carcinogen that inhibit chain elongation in operating replicons, the inhibition of replicon initiation can still be observed (49). However, the magnitude of the inhibition has not been determined after these toxic doses. It is conceivable that a large percentage of replicons delay initiation after exposure to carcinogen with a time-course that exceeds that seen after lower sub-lethal doses. Waters (64) has shown that cyclobutane pyrimidine dimers are repaired at the same rates in replicating and non-replicating DNA of human fibroblasts. Thus, there does not appear to be a preferential repair of replicating DNA. Mitchell et al. (65) have shown that the 6—4 PyC dimers are repaired much more rapidly than the cyclobutane dimers and arguments presented below suggest that the PyC dimers may represent more effective blocks to chain elongation than do the cyclobutane dimers. Consequently, until the repair of the 6—4 PyC lesions is determined for replicating and non-replicating DNA, it is not possible to determine the restrictions on replicon initiation imposed by excision repair capacity. It is clear that the inability to repair UV-induced photoproducts is associated with a significant delay in the recovery of initiation of replicons (48). Thus, it may be that initiation of replicons is linked to the removal of pathways of DNA excision repair of DNA lesions that block RNA transcription or interrupt topoisomerase activity. The recently established preferential repair of transcriptionally active genes (66) suggests that mutagenesis in these genes will occur only when carcinogen exposure closely precedes or coincides with their time of replication in the S phase.

**Mechanisms of elimination of discontinuities**

Having reviewed the mechanisms of inhibition of DNA replication by agents that produce non-instructive or steric distortions of DNA templates, we can now address the potential mechanisms of elimination of the discontinuities in daughter strands. Pathways of post-replication repair that will eliminate the discontinuities are shown in Figures 5 and 6. The simplest pathway involves insertion of a randomly chosen base opposite the template lesion so as to circumvent the non-instructive lesion. The inserted base is not directed by information in the hydrogen-bonding region of the altered template bases and so DNA polymerase has a 25% probability of inserting the correct base. This mechanism of post-replication repair will be mutagenic leading to base substitutions 75% of the time. If the base that is inserted at non-instructive template lesions is specifically selected [e.g. adenosine (67)], then the probabilities of mutagenesis will be determined by the identity of the non-instructive lesion. For example, removal of uracil by glycosylase action produces a potentially mutagenic apyrimidinic site. If the uracil originated by deamination of cytosine, the insertion of adenosine is a mutation. If uracil was incorporated during DNA synthesis after deamination of deoxycytidine triphosphate precursor, then insertion of adenosine is not a mutation. The cyclobutane thymine—thymine (TT) dimer may be a non-instructive template lesion that impedes polymerization (68, 69). Insertion of A at this lesion will circumvent the block and eliminate discontinuities without producing a change in the primary sequence of DNA. The failure of plasmid-based mutagenesis assays to detect mutations at sites of cyclobutane TT dimers (70—72) suggests that this mechanism of lesion bypass may be favored in UV-irradiated human cells.

Another pathway of post-replication repair is based on the observation that many spontaneous deletions in the lac gene of *Escherichia coli* occur where the template strands can form stable hairpin loops, or palindromic cruciforms (73). It is conceivable that the single-stranded DNA template at the site of a blocked growing point can form into a hairpin which contains the template lesion as illustrated in Figure 5. Continuation of synthesis of the nascent strand will occur when a sufficient number of hydrogen bonds are formed between the primer terminus region of the
Fig. 6. Recombination pathway of post-replication repair. Parental template strands are shown as white ribbons and nascent daughter strands are shown as black ribbons. Interaction between a daughter-strand gap at the site of template damage (A) and the Rec A-like recombinase enzyme may lead to paranemic invasion of the sister duplex by the damaged template strand (B). Renaturation of the 3' OH primer terminus with denatured nascent DNA relieves the blockage of chain elongation and leads to formation of a replication intermediate composed of two Holliday junctions separating regions of DNA duplex composed of both parental strands and both daughter strands (C,E). Resolution of these intermediates by the various combinations of cutting at each of the junctions will generate either a recombination patch (F) or a sister-chromatid exchange (D). Note that in the sister-chromatid exchange the sequence of white parental strand and black daughter strand that is seen at the cross-over site proximal to the replication fork is reversed after the distal cross-over site.

The third pathway whereby discontinuities in nascent strands may be eliminated is recombinational exchange which appears to be the principal mechanism of post-replication repair in *E. coli* (25). Regions of single-stranded template may be substrates for the activity of enzymes involved in homologous recombination (Figure 6). Activities have been recently isolated from human cells (76—78) which perform a Rec A-like function, the invasion of homologous duplex by ssDNA with formation of a D-loop. In this case the region of template containing the blocking lesion is annealed to its original (i.e. pre-replication) partner in the duplex. It is possible to create a structure which has a thermodynamic stability similar to that of two sister duplexes but in which a region of each duplex is composed of either both parental strands or both daughter strands (Figure 6). Resolution of this recombination-like intermediate which contains two Holliday junctions (79) will have two outcomes (80). Cutting of both junctions along either the horizontal or the vertical axes will create one daughter duplex with a patch of parental strand inserted into the daughter strand and another daughter duplex with a patch of daughter DNA inserted into the parental template strand. Cutting of one of the junctions along the vertical axis and the other along the horizontal axis resolves the intermediate but generates a sister-chromatid exchange. This recombinational pathway of post-replication repair appears to be capable of error-free elimination of discontinuities.

Which pathway of post-replication repair to use?

These three mechanisms of elimination of discontinuities (non-directed insertion, deletion/dislocation, and recombination) encompass a broad range of disparate biochemical processes utilizing quite different enzymatic activities. What elements might favor the use of one pathway over the other? Several factors would appear to be important. One is the precise structure of the lesion in the template. For example, the principal UV-induced photoprodut, the cyclobutane TT dimer, may produce less distortion of the template than the 6—4 PyC dimer (81). No hydrogen-bonding information is lost in the cyclobutane TT dimer (82). Moreover, the alignment of the two pyrimidine rings may not be incompatible with the activity of DNA polymerase, especially within replication complexes in which accessory factors are present. It should be noted here that the precise crystal structure of any pyrimidine dimer embedded in duplex or ssDNA has yet to be determined. Model-building studies have been performed to examine the structure of pyrimidine dimers embedded in DNA (81,83). When DNA was constructed around the dimer, using molecular coordinates for the dimer that were established from X-ray crystallographic analysis of thymine—thymine cyclobutane dimers, substantial distortions of DNA helical structure were obtained (83). However, when dimers were...
embedded within B-form DNA without imposing the crystal structure (which may apply only to dimers of the free thymine base), a less substantial and more local distortion of structure at the site of the dimers was generated (81).

Although the prevailing dogma holds that cyclobutane TT dimers constitute impediments to DNA polymerase and in vitro DNA synthesis assays using naked primer templates and purified enzymes indicate that DNA polymerase does at least pause at these dimers (68,69), the biological data argue strongly for a majority of UV-induced dimers being rapidly bypassed by DNA polymerase in normal human fibroblasts. Two lines of evidence support this conclusion. One is the numerical density of lesions required to interfere with fork progression at DNA growing points. The D_{50} for inhibition of DNA chain elongation in diploid human fibroblasts were one lesion/replicon for BPDE and six lesions/replicon for UV (28, J.C.Boyer, W.K.Kaufmann and M.Cordeiro-Stone, unpublished results). If the BPDE adduct represents an extremely effective impediment to polymerase, then cyclobutane dimers are only about one-sixth as effective. Note also that the 6—4 PyC dimer, which can be expected to interfere with polymerase activity, appears to be formed with about one-tenth the frequency of the cyclobutane dimer (71,84). A second observation also suggests that normal human fibroblasts are capable of rapid transdimer synthesis. It has been determined that xeroderma pigmentosum (XP) variant fibroblasts, which are generally considered to be defective in post-replication repair (26, 27,48,51,85), are three- to four-times more sensitive than normal fibroblasts to inhibition of fork progression and strand growth by UV (28,48,85). Because replication by these cells in the absence of damage appears to be normal (26,48), and their excision repair ability appears to be normal (27,48,86), it has been proposed that DNA synthesis is inhibited in the XP variant cells at certain lesions which have no apparent effect on DNA synthesis in normal human fibroblasts (48,85). The lesions that block replication in the XP variant are almost certainly cyclobutane pyrimidine dimers (87) and, so, the inescapable conclusion is that 67—75% of these dimers are ineffective blocks to DNA replication in normal human fibroblasts. It seems as though the XP variant lacks a gene product that allows replication to proceed through cyclobutane pyrimidine dimers in template strands without substantial delay. XP variant fibroblasts display hypersensitivity to mutation and transformation by UV (88—91). If interruption of DNA polymerase activity by template lesions is responsible for mutagenesis and transformation, then the available data suggest that XP variant cells are defective in an error-free mechanism of post-replication repair. Could the XP variant utilize a DNA polymerase holoenzyme complex that fails to insert A across from cyclobutane TT dimers? Some XP variant strains do not appear to be appreciably hypersensitive to BPDE-induced template damage (85,91) and thus the replicational deficiency is not generalized to all blocking lesions in template strands.

In the 6—4 PyC dimers the hydrogen-bonding region of the 3'-cytosine is partially destroyed and is removed from the space that it would occupy in a ssDNA template (81,83). Arguments suggesting that these lesions are primarily responsible for UV mutagenesis in E.coli have been advanced (92). An additional observation suggests that 6—4 PyC dimers are more of a problem to human cells than are cyclobutane pyrimidine dimers. As previously mentioned, Mitchell et al. have shown that the 6—4 PyC dimers are repaired in normal human fibroblasts at a rate of 4- to 5-times that of the cyclobutane dimers (65). It evidently benefited dermal fibroblasts to repair the 6—4 lesions rapidly to have evolved such an efficient repair capacity. This also implies that the risks of unrepaired 6—4 PyC dimers outweigh the risks of unrepaired cyclobutane dimers.

This discussion emphasizes the importance of lesion structure in considerations of mechanisms of post-replication repair. Along these same lines, it was mentioned that the BPDE—N2 guanosyl DNA adduct appears to represent an efficient impediment to DNA polymerase. This might be explained by the large size of the polynuclear aromatic ring structure which should sterically interfere with movement of polymerase along the template strand (Figure 2). However, in ssDNA the bases have free rotation about the phosphodiester bonds. Rotation of the adducted guanosyl residue may relieve the steric impediment with the cost of loss of hydrogen-bonding information. In this situation the non-instructive abasic site may be circumvented by insertion of a nucleotide to span the non-coding lesion. The non-directed insertion mechanism of post-replication repair may be preferred over the deletion/dislocation mechanism, for a base-substitution mutation need not be pathological for the cell. Non-directed insertion may be a stochastic event occurring with a constant low probability per non-coding lesion. Failure to insert increases the probability that deletion/dislocation or recombination pathways are used.

Another factor that should affect mechanisms of post-replication repair is the sequence context of the blocking lesion. In the deletion/dislocation pathway, impediments may be overcome when the region of template containing the lesion folds back upon itself forming a hairpin loop. Thus, the ability of the damaged region of DNA to form a loop and then to re-anneal to the primer terminus beyond the loop will determine whether this mechanism is used for post-replication repair. Palindromic sequences may be quite abundant in human cells (93). Palindromes are important signal sequences in the immunoglobulin gene rearrangements that produce antibody diversity (94), they may be controlling sequences in transcription of inducible genes (95), they appear at hotspots for amplification-associated rearrangements of the adenylate desaminase gene in Chinese hamster cells (96), and they have been associated with deletions in the human β-globin gene cluster (97). It will be interesting to determine whether there is any overlap between DNA metabolic pathways that are important in chemical or radiation carcinogenesis and pathways of developmentally-programmed gene rearrangements, as has been suggested (98). Sequence analysis of carcinogen-induced deletion mutants should establish the relationship between DNA primary structure and post-replication repair.

Post-replication repair of damaged episomes

With the development of methodologies of DNA sequencing and molecular biology it is now possible to determine directly the alterations of DNA sequence that occur when human cells replicate damaged episomal DNA molecules (70—72,99,100). As previously mentioned no mutations were observed at sites of cyclobutane TT dimers in episomes (70—72). G to A transitions were observed at TC and CC sites that can form the 6—4 PyC dimers as well as cyclobutane dimers. This result implies that deoxyadenosine was inserted by DNA polymerase to bypass non-instructive template lesions. The majority of base-substitution mutations that were produced in episomal DNA after treatment with BPDE were GC—TA transversions consistent with insertion of deoxyadenosine to bypass the template lesion (100). A minority of GC—CG transversions were observed suggesting that syn-anti isomerization of the BPDE—N2
guanosine adduct may also relieve the blockage by allowing mis-instructive formation of hydrogen bonds with an incoming deoxyguanosine triphosphate precursor (101, 102). Deletions of genetic material were observed in ~20% of recovered mutants that were induced in the episome by BPDE (100) and in 30% of episomal mutants induced by ethylN-nitrosourea (99). When the human chromosome 11 was replicated in Chinese hamster ovary cells after exposure to benzo[a]pyrene, 98% of mutants that lost a non-essential surface marker encoded in chromosome 11 had also lost one or more linked marker genes, suggestive of genetic deletion (103). Many of the dihydrofolate reductase-deficient mutants that were induced by acetoxy-acetylaminofluorene (AAAF) in Chinese hamster cells also contained deletions of genetic material (104). The C8-deoxyguanosine-AAF adduct in DNA appears to represent an effective block to DNA polymerase (69). Consequently, although non-directed insertion can be identified as a pathway of post-replication repair, other pathways that lead to deletions also appear to be utilized.

Recombination revisited

These concepts of post-replication repair consider the blocked primer terminus and the exposed ssDNA template to be unique biochemical substrates for nuclear enzymes involved in DNA metabolism. Thus, in the recombination mechanism, the RecA-like protein that catalyzes strand invasion of homologous duplex is envisaged to recognize certain structures of DNA which are produced when replication complexes encounter blocking lesions in the template (Figure 3). Initial studies that were designed to identify the recombination of damaged template DNA with newly synthesized daughter DNA (105, 106) suggested that 3–25% of lesions were recombined into daughter strands. However, because asynchronous cell cultures were examined it was possible that a large fraction of these lesions were actually formed in daughter DNA that had already been made at the time of irradiation (107). Using the highly sensitive alkaline elution assay, which could detect a very low level of recombination, Fornace demonstrated that a small fraction of UV-induced pyrimidine dimers, that were introduced into the DNA template when human cells were in G1, eventually were recovered within newly synthesized daughter strands consistent with a recombinational event (108). The fraction of dimers that was recombined was 1–4% suggesting that this pathway did not represent the principal one used in damaged human cells. However, given the arguments already advanced that blocking lesions may represent but 10–16% of the total yield of UV-induced pyrimidine dimers, the data of Fornace (108) imply that in excision-repair-defective XP cells as many as 40% of the blocking lesions in template strands actually may be involved in recombination with daughter strands. It is interesting that only a small fraction of these recombinational events generate sister-chromatid exchanges. Repair-deficient XP fibroblasts may accumulate 5–15 induced sister-chromatid exchanges per cell after a UV-fluence that produces one dimer in 10^8 daltons (109). With an average genome size of ~4 x 10^{12} daltons, this represents ~1–4 exchanges per 10^{12} daltons or one per 2500–10,000 pyrimidine dimers. Consequently, it is necessary to envisage a restricted subset of at most 1% of recombination intermediates which is resolved as a sister-chromatid exchange with the majority being resolved by formation of the recombination patch (Figure 6).

Chromatid-type chromosome aberrations may be the result of the failure of post-replication repair pathways to eliminate discontinuities

It should be considered that some substrates cannot be modified by any of the above-mentioned mechanisms of post-replication repair so that the discontinuities in daughter strands are more or less permanent. One such substrate has been described by Park and Cleaver (26) and further explored by Painter (44) in which replication forks in adjacent replicons become permanently blocked leading to the formation of a long-lived unreplicated region. This structure will produce considerable problems at mitosis for it should promote non-dysjunction. If such a substrate encounters a single-strand-specific endonuclease, double-strand breaks will be formed (110). Double-strand breaks have been observed in UV-irradiated S phase cells (111), and significantly greater numbers of these breaks occurred in repair-deficient XP cells suggesting that they were not a consequence of overlapping DNA excision repair sites. Double-strand breaks have been implicated in the formation of chromosomal aberrations (11, 12). Moreover, conditions which delay the sealing of DNA excision repair patches also have been shown to enhance the formation of chromosomal aberrations (11, 12). Blocked growing points are quite similar in structure to open repair patches and might be expected to have similar cytogenetic consequences. Based upon these considerations, it seems reasonable to propose that the formation of chromatid-type chromosomal aberrations is the consequence of a failure of post-replication repair pathways to eliminate discontinuities before they lead to double-strand breaks. The action of single-strand-specific endonucleases at blocked growing points can be expected to yield intermediates that lie within a DNA metabolic pathway leading to chromatid-type chromosomal aberrations. A chromatid break may result from the formation of an irreparable double-strand break at a single blocked growing point. A chromatid gap may be generated by two unrepaired double-strand breaks flanking long-lived unreplicated regions of DNA. Inter-chromatid exchanges should be a consequence of the interaction of two double-strand breaks (or their repair complexes) on different chromatids.

Inducible post-replication repair?

A final element of discussion must be devoted to the question of inducible post-replication repair. The concept that damaged cells may induce gene products which affect the process of post-replication repair originated with the demonstration of the SOS pathway in E.coli (25) which includes numerous inducible genes. Evidence for the operation of inducible pathways in human cell post-replication repair has been derived principally from split-dose experiments. A small priming dose of UV or AAAF was shown to reduce the effect of a second larger dose of AAAF (52) or to increase the rate of recovery of DNA synthesis after a second dose of UV (112). This approach has also been applied to analysis of viral reactivation in diploid human fibroblast strains (113). If damage-inducible processes occur to aid in post-replication repair of viral DNA, the extent of enhanced DNA synthesis may be only in the order of 2-fold in normal human fibroblasts (113). This is in contrast to E.coli in which the inducible pathway increases reactivation of bacteriophages by 10-fold (25). It should be recognized that the process of cell proliferation involves the induction of numerous gene products
(114) including enzymes directly involved in DNA metabolism such as DNA polymerase α (115), DNA ligase (116), DNA polymerase δ accessory protein (117) and DNA topoisomerase II (54). Given the demonstrated effects of carcinogen on replication operation, it is apparent that damaged S phase cells may experience dramatic temporal alterations in the balances of gene products which effect the replication of chromosomal lengths of DNA. Only when these alterations are sufficiently characterized and their interactions are understood, can the theoretical concept of inducible post-replication repair be subjected to experimental analysis. Moreover, the demonstrated alterations in base structure that follow carcinogen treatment are sufficient in themselves to induce mutagenesis and related genetic alterations without having to invoke as causative elements inducible gene products, other than those that occur as a natural consequence of the signal to proliferate.

Summary
In summary two mechanisms of inhibition of DNA replication have been identified in carcinogen-damaged human fibroblasts, reduced initiation of DNA synthesis in replicon clusters and interference with template-directed DNA polymerase activity. The inhibition of replicon initiation interacts synergistically with DNA excision repair to reduce the probability of replication through damaged template. Within operating replicons DNA chain elongation appears to be inhibited by lesions which distort the structure of the DNA template so that polymerase does not see hydrogen-bonded deoxyribonucleoside triphosphate precursors at growing points or it is sterically prevented from polymerization and movement along the template. Interference with polymerase activity perpetuates discontinuities in the nascent daughter strands and regions of single-stranded template may remain exposed for considerable intervals. The structures of DNA at blocked growing points may be modified by proteins or by thermodynamic stabilization so as to allow elimination of the discontinuities. Some of these pathways of elimination of discontinuities are error-free and some are error-prone. The error-prone pathways of post-replication repair are believed to be responsible for the pathologic sequelae of DNA replication on damaged templates including base-substitution and deletion mutagenesis. Chromatid-type chromosome aberrations are postulated to result from endonuclease attack on ssDNA at blocked growing points leading to irreparable double-strand breaks.

References


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