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RELATIONSHIP OF DNA LESIONS AND THEIR REPAIR TO CHROMOSOMAL ABERRATION PRODUCTION[1]

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Running Head: Chromosomal Aberrations

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ABSTRACT

Recent work on the roles of specific kinds of DNA lesions and their enzymatic repair systems in the production of chromosomal aberrations seems consistent with a simple molecular model of chromosomal aberrations formation. Evidence from experiments with the human repair-deficient genetic diseases xeroderma pigmentosom, ataxia telangiectasia and Fanconi's anemia is reviewed in the light of the contributions to aberration production of single and double polynucleotide strand breaks, base damage, polynucleotide strand crosslinks and pyrimidine cyclobutane dimers.

INTRODUCTION

Perhaps one of the most notable developments in cytogenetics of the past decade is the integration of the ideas and techniques of molecular biology and genetics that has occurred over the past few years. Though the production of chromosomal aberrations by radiation has of course been studied intensively from the biophysical point of view for over four decades (30), and by chemicals from the biochemical for over three (26), and despite occasional perceptions of the implications of DNA synthesis and repair biochemistry (see for example (60) or (12)), I think it fair to state that recent work on the roles of specific DNA lesions and their repair has led to a real breakthrough in our understanding of the molecular mechanisms involved in what has come to be called clastogenesis. Reviewing such a rapidly developing field is always difficult, and the result never completely up to date, so what follows is necessarily selective rather than comprehensive, and certainly presents a personal and not necessarily generally accepted view of the topic.

A striking consequence of recent cytogenetic developments is that it is now possible to interpret chromosomal aberration production in terms of general molecular models in much the same manner as it earlier became possible to interpret gene mutation in molecular terms. Any specific model may always, of course, prove inadequate in the light of new data, or even totally wrong. Nevertheless, the testing of such models is productive, and it has been the study of the cytogenetic results of the introduction of specific lesions into DNA at specific points in the cell cycle and the study of the influence of specific DNA repair systems using genetic repair deficiencies, repair poisons such as caffeine, or other manipulations of repair capacities such as "liquid

holding recovery" that has led to our recent rapid progress. The parallel with molecular genetics is striking.

A MODEL

While other models are possible, and many features have not as yet been rigorously proven, I believe that the very simple one we formalized some years ago (3) still constitutes, with a few additions, an acceptable framework within which to understand the molecular mechanisms involved in chromosomal aberration production and the roles of specific DNA lesions and their enzymatic repair systems. Briefly, it contains the following elements.

First, the eukaryote chromosome is considered to be mononeme, each pre-replication (G_1) chromosome or post-replication (G_2) chromatid containing a single DNA double helix. The double helices of each G_2 chromatid are of course comprised of one "old" polynucleotude strand from the G_1 molecule and one new one synthesized using the old one as a template (62). Whether there is just one DNA molecule or a series joined by "linkers," with respect to aberration production, the chromosome or chromatid behaves empirically as though the DNA runs continuously from one end to the other.

Second, whatever intermediates there might be, the ultimate target for aberration production is the DNA. Changes in other chromosomal constituents will result in aberrations only if they lead in turn to alterations in DNA.

Third, aberrations consist of polynucleotide chain breaks and of recombinations between their broken ends. Almost by definition, a chromosome or chromatid break involves a DNA double strand break. Aberrations of the chromosome type, with both daughter metaphase chromatids affected at the same distance from their ends or centromere, result from replication of double strand

breaks or of "illegitimate" products of rejoining between their broken ends. Aberrations of the chromatid type, affecting (with one exception) only one of the two daughter metaphase chromatids, arise from double strand breaks in already replicated (S and G_2 phase) DNA. Achromatic lesions (or "gaps"), whether considered true aberrations or not, are manifestations of breaks and possibly other DNA alterations involving only one polynucleotid strand, and thus consititute "half chromatid aberrations." Again by definition, half chromatid exchanges are between single polynucleotide strands, though it seems likely that the rejoining involved is actually between double strand breaks occurring in palindromic or reverse tandem complementary repeat base sequence regions of single polynucleotide strands while paired in the "hairpin" configuration. Figures 1 and 2 illustrate these ideas schematically.

Fourth, double polynucleotide strand breaks may arise directly, but are frequently generated secondarily as a consequence of normal DNA synthesis, of enzymatic DNA repair processes, or through the action of specific nucleases not necessarily related to DNA repair. Single polynucleotide strand breaks present in template strands during local DNA synthesis are duplicated in the nascent strand, thus yielding double strand breaks in the daughter molecule; other lesions can also interfere with the template function and result in the "synthesis" of single strand breaks in the nascent strand. Single strand breaks can further be converted to double strand breaks by either specific repair endonucleases or endonucleases specific for single-stranded DNA. Some of the possibilities are illustrated schematically in Fig. 3.

Fifth, the process of rejoining of double strand breaks probably arises through precisely the same mechanisms as are now so widely employed in the deliberate construction of recombinant DNA molecules for cloning, namely, the

creation of single stranded "sticky" ends by specific exonuclease activity like that of lambda exonuclease or DNA polymerase I, the annealing of sticky ends posessing sufficient complementarity, trimming or filling out of the free ends by exonuclease and/or polymerase activity, and final closing of the chains by ligase activity. This is shown in Fig. 4. Essentially this mechanism was proposed by J. H. Taylor many years ago [60]; some of the evidence supporting it, particularly that relating to repetitive DNA base sequences, was recently summarized by Chadwick and Leenhouts [9].

It seems quite possible that the temporal decrease in the capacity of "old" chromosome breaks to rejoin with "new" ones induced later on in the cell cycle, which gives rise to the well-known fractionation effect for exchange aberrations, as well as the intriguing ability of chromatid breaks occuring in sister chromatids (isochromatid deletions) to rejoin with each other in sister union configurations may also arise through a similar mechanism involving the production and then annealing of stickey ends. Cavalier-Smith [8] some time ago proposed that chromosome ends or telomeres might consist of palindromic base sequences as an explanation of how the synthesis of the 5' ends of linear DNA molecules might be accomplished. The terminal hairpin configuration is ligated in this model so that there are literally be no free polynucleotide strand ends except while synthesis is actually in progress. A similar annealing of sticky ends of chromosome breaks containing palendromic base sequences would lead to the creation of the telomeric configuration as shown in Fig. 5, with the consequence that the breaks would no longer be available for interactions with other breaks. A similar process, also illustrated in Fig. 5, and akin to that proposed by Chadwick and Leenhouts [9], would also result in isochromatid sister unions.

Simple as is this model of chromosomal aberration production, it appears adequate at least for the present. While the idea that established molecular mechanisms can satisfactorily explain cytogenetic phenomena is a relatively new one to many cytogeneticists, current information on the effects of both specific DNA lesions and their enzymatic repair mechanisms appears to support it, as I shall attempt to show.

STRATEGIES

Several approaches to the elucidation of the roles of various DNA lesions and their repair mechanisms have been employed, including the introduction of specific lesions into cells' DNA at specific points in their cell cycle, the comparison of responses of cells differing genetically in DNA repair capacities, selective removal of specific DNA lesions after treatment with agents inducing more than one type of DNA lesion, and the use of agents or conditions that increase or decrease the effectiveness of various DNA repair processes. These are powerful strategies, as a few examples will show.

Introduction of Specific Lesions. The simplest way to relate aberration production to specific kinds of DNA lesions is to induce them in a cell at a specific point in its cell cycle and then to examine the consequence at the next mitosis. The requirement that the lesions be introduced at a known time stems partly from the fact that some DNA lesions produce aberrations only when the lesion-bearing DNA is used as template for the synthesis of new DNA. In addition, some lesions result in one kind of aberration at one point in the cell cycle and quite another, or even none at all, when introduced at a different point. Ionizing radiation, for example, produces chromosome type aberrations in G_1, chromatid types in S and G_2, and subchromatid exchanges in prophase, while UV

light probably usually produces only chromatid types and these only (or mainly) if the cells synthesize new DNA.

Unfortunately, most agents produce DNA lesions of more than one type. Again, ionizing radiation is a good example, for it produces both single and double polynucleotide breaks as well as various kinds of base damage, all of which, it appears, can give rise to aberrations. However, a few systems do appear to produce only one, or at least mainly one type of lesion, and their study has proven profitable. An example is the induction of single polynucleotide strand breaks by photolysis of DNA in which 5-bromodeoxyuridine has been substituted for thymidine (2).

Lesion Removal. It is sometimes possible to selectively remove one specific type of DNA lesion from among a variety that may have been induced by treatment with a given agent, and thus to elucidate its role in aberration production by observing what cytogenetic effects no longer result following its removal. For example, cells from some (but not all) eukaryotes possess a photoreactivating enzyme which in the presence of visible light of the right wavelength efficiently monomerizes pyrimidine cyclobutane dimers. By such selective elimination of this lesion from the DNA of cells treated with UV light we were able to demonstrate the role of such dimers in the production of chromosomal aberrations (17). Comparisons of the effects of related agents differing in their ability to induce one specific class of lesion from among a spectrum of lesions also constitutes a valuable "subtraction" approach, as was shown for example by Sasaki in his study of the sensitivity of Fanconi's anemia cells to crosslinking agents and to a monofunctional derivative (49, 51).

Genetic Repair Defects. A classical stratigem in microbial mutation research has been the identification of specific genetic DNA repair deficiencies

and the study of their influence on the induction of mutation. The specificities of repair systems for particular lesions or families of lesions has allowed important insights into the nature of mutagenesis itself and the molecular mechanisms involved in the production of mutations by particular DNA lesions. Several genetic repair defects have now been identified in humans, and study of their influence on chromosomal aberration production by various clastogens has begun. Perhaps the best known is xeroderma pigmentosum (XP), a defect in the excision repair of pyrimidine cyclobutane dimers and certain other DNA lesions. A number of studies have shown that UV light induces more chromosomal aberrations in XP than in normal human cells in tissue culture (for example [38]). XP cells are also more sensitive to some chemical mutagens; for example as Sasaki [48] has shown for aberration production by 4-nitroquinoline 1-oxide.

Two other human genetic disorders, ataxia telangiectasia (AT) and Fanconi's anemia (FA), appear to involve specific DNA repair deficiencies, and are being used currently in chromosome aberration studies, as I will describe presently.

Because species also differ in DNA repair capacities, similar comparisons are possible between the cytogenetic responses of cells of these species. For example, cells from murine rodents excise UV-induced dimers from their DNA only poorly, while those from normal humans excise them rapidly. As would be expected, treatment with UV light in G_0 or G_1 induces aberrations much more efficiently in Chinese hamster tissue culture cells than in human lymphocytes [4, 34, 38].

Repair Modification. Modification of the abilities of cells to repair DNA damage is a classical method of demonstrating the existence of systems capable of repairing the damage and their influence on mutation induction, as exemplified by "liquid holding recovery," or the use of caffeine as a repair "poison" in bacteria. The same strategies have been employed in the study of chromosomal aberration production, as for example in recent experiments on the influence of holding cells in the confluent state on aberration fields (14) or in numerous studies of the influence of caffeine on yields of chromosomal aberrations induced by a variety of mutagens (27).

SPECIFIC LESIONS

Though there remain gaping holes in the picture, there is already enough evidence to allow us to be fairly certain regarding the roles of certain DNA lesions or classes of lesion in aberration production, and to begin, at least, to catalog them and the influences of their specific repair systems.

Single Polynucleotide Strand Breaks. The role of single strand breaks in aberration production is clearly complex. Such breaks are of course one of the consequences of exposure to ionizing radiation, and apparently of treatment with certain chemicals such as the chemotherapeutic agents bleomycin and neocarzinostatin as well (35, 40). From their study of the cytogenetic effects of treatment with the base analog 5-fluorodeoxyuridine, Taylor, Haut and Tung (61) deduced that S phase cells so treated arrived at metaphase with gaps left in the nascent polynucleotide strands, and that these gaps could appear as achromatic lesions. Precocious condensation of S phase chromosomes in which the nascent strand is still incomplete also yields metaphase chromosomes with achromatic lesions (24). Our own study of the effect of photolysis of 5-bromodeoxyuridine incorporated into only one of the polynucleotide strands of

each DNA double helix yielded similar results, and further led us to conclude that single strand breakage could also result in chromatid and isochromatid aberration production, processes we postulated to arise from single strand nuclease activity, normal DNA synthesis, and the operation of a recombinational DNA repair mechanism (2), all of which would, as already noted, result in the conversion of single strand breaks into double ones. Interestingly enough, Natarajan and Obe (36) have recently reported an increase in induced chromatid aberration yields in cells treated with exogenous single strand nuclease in the presence of inactivated Sendi virus. Single polynucleotide strand breaks are quite rapidly repaired by most cells (55), so one might expect that achromatic lesion yields would be greatest for cells irradiated in late G_2 and that they would decrease quite rapidly for progressively earlier irradiations as more time was allowed for repair prior to their becoming "caught" by chromatin condensation in preparation for cell division. This is, of course, precisely what happens.

From the classical observation that treatment with ionizing radiation during the G_1 phase of the cell cycle normally does not result in the production of chromatid type aberrations, I conclude that single strand breakage is not normally an important contributor to chromosomal aberration production during this phase of the cell cycle (3). However, Wolff (64) and others have shown that if G_1 cells are irradiated very close to the G_1-S border some chromatid type aberrations can result. It seems likely that the reason chromatid aberrations are not usually observed following G_1 irradiation is because the repair of single strand breaks is usually very rapid, so that none are left as such by the time the S phase commences, though some may, of course, be converted to double strand breaks by single strand endonuclease activity, and thus contribute to chromosome type aberration production. However, if the lesions are induced very shortly

before the beginning of S, such repair would still be incomplete, and would produce chromatid aberrations in the same manner as if they were induced by S phase irradiation.

Two rare human recessive genetic disorders, ataxia telangiectasia and Fanconi's anemia, previously reported to be characterized by an abnormal chromosomal sensitivity to ionizing radiation of their peripheral lymphocytes when irradiated in G_0 [19,20] have both been found to be radiosensitive when irradiated in G_2, exhibiting increased yields of both achromatic lesions and chromatid deletions [7, 41, 57]. Such a finding suggests the possibility of a strand break repair deficiency. Several investigations, however, have failed to demonstrate any such deficiency on the biochemical-biophysical level, at least in AT [58, 63].

Double polynucleotide strand breakage. As noted earlier, given a mononeme model for the eukaryote chromosome and the overwhelming evidence that DNA is at least the principal target in clastogenesis, direct induction of double strand breaks must, almost by definition, constitute chromosome or chromatid breakage. Furthermore, those agents known to induce double strand breaks directly, or at least promptly, produce chromosome type aberrations; those that do not do not appear to produce prompt double strand breakage either. Though this is true for most biological effects of ionizing radiation, it is perhaps worth noting that the well known observation that chromosomal aberrations are produced with greater efficiency per unit dose with increasing linear energy transfer is paralleled by an increasing DNA double strand breakage efficiency [10, 25], a finding at least consistent with their postulated role in aberration production.

Though there has been some confusion on the point, it now seems clear that there is repair of radiation-induced double strand breaks in eukaryotes

(11, 21, 46). Resnick (45) has proposed a recombinational model of double strand break repair through the formation of heteroduplexes with intact homologous molecules, and he and Martin (46) have verified some of the predictions of the model in yeast, as have Krasin and Hutchinson (28) in E. coli. However, it remains to be seen whether this is the only, or even a predominant, mode of repair in the cells of higher eukaryotes.

Chadwick and Leenhouts (9) have recently proposed the recombinational double strand break repair model of Resnick as an explanation of certain features of chromosomal aberration production by ionizing radiation. Though their theory that chromosomal exchange aberrations must arise from single double strand breaks, rather than from two independently induced breaks seems to me unnecessary for reasons some of which have been discussed by Savage (52), the proposal remains an interesting possibility. We proposed a recombinational repair process as an explanation for the ability of lesions in one polynucleotide strand to "spread" and result in the formation of isochromatid deletions (4) Chadwick and Leenhouts have shown that not only could the Resnick mechanism account for "spreading", but that it could also, if occurring in the region of a palendromic base sequence, account for the sister union phenomenon.

Among the human genetic diseases conferring sensitivity to mutagens, ataxia telangiectasia has been investigated to determine whether cells from these patients might be deficient in their ability to repair DNA double strand breaks. Taylor, et al., (58) and Lehmann and Stevens (31) both report, however, a normal double strand break repair capacity for such cells.

The earlier reported G_1 chromosomal radiosensitivity of lymphocytes from AT patients (20) has also been reported by Taylor (57), though the increased yield he reported in G_0-irradiated AT lymphocytes was, unlike that seen by

Higurachi and Conen, largely in the fragment (i.e., deletion) class rather than in rings and dicentries. Though Taylor argues that the techniques used to measure double strand break repair might not be sensitive enough to detect a deficiency sufficient to account for it, the G_0 chromosomal radiosensitivity in AT lymphocytes nevertheless seems to be in conflict with the apparently normal double strand break repair in AT cells, and the idea that double strand DNA break production is the basic mechanism by which aberrations are formed, unless, possibly, the DNA of AT cells is simply more easily broken by ionizing radiation. The available data, however, do not suggest an increased double strand break yield (31, 58). We have therefore reinvestigated the reported AT G_0 lymphocyte sensitivity (6), employing a 5-bromodeoxyuridine substitution-differential staining technique (65) to allow both the scoring of only cells unequivocally in their first post-irradiation mitosis and also the determination of whether there are differences in cell proliferation dynamics (53) between AT lymphocytes and control lymphocytes from normal individuals. Preliminary experiments showe that the addition of 5-bromodeoxyuridine to lymphocyte cultures to a level of 25 μM following irradiation neither increases aberration yields nor produces appreciable changes in proliferation dynamics.

The preliminary results from two such experiments involving four different AT patients and three normal controls are summarized in Table 1. Neither the two experiments nor the results from fixations at either 72 or 96 hours appears to differ appreciably, so they are pooled in the Table for the purpose of illustration. Two features of the chromosome type aberration yields are immediately apparent: first, the yields of rings and dicentrics are no higher in the AT cells than the controls, and second, there is a large excess of deletions in the AT cells. Though I shall return to this point in my discussion of

DNA base damage, it is necessary to note here that AT cells display the peculiarity of being susceptible to the induction of chromatid type aberrations by G_0 or G_1 irradiation; this is seen in the Table. Because isochromatid deletions and chromosome type deletions are indistinguishable except when sister union occurs (an event that appears to be relatively rare in human cells) the aberrations scored as chromosome deletions actually include an unknown number of isochromatid deletions as well. If their frequency is increased in G_0-irradiated AT cells as is the frequency of other chromatid types, as seems reasonable, then much if not all of the apparent increase in chromosome deletions might disappear were we but able to distinguish between them.

When examined in this light, the reports of G_0 AT lymphocyte radiosensitivity of Taylor, et al. [57, 59] also suggests that in fact the difference from normal cells may be accounted for entirely by the chromatid aberration increase. As noted already, the ring and dicentric yields he observed were not much, probably not statistically, greater. Futhermore, Taylor [57] reports that many of the exchange aberrations seen in both normal control and AT lymphocytes lacked the expected acentric fragments, and the controls more often than the AT cells, particularly at the higher doses. This suggests to me that second post-irradiation mitoses were included in his sample, and with higher frequency in the normal than in the AT cultures, though Taylor argues otherwise.

We therefore conclude that the reported G_0 sensitivity of AT lymphocytes is not in fact a sensitivity to the induction of chromosome type aberrations. In the earlier experiments no means of excluding second or later post-irradiation mitoses from the sample scored was available, and it seems likely that the difference can in part be attributed to the inclusion of such cells, which would have a lower aberration frequency because of losses at the first mi-

tosis. Not only do the data of Taylor on exchanges lacking fragments suggest this, but our data on the relative frequencies of first, second and third or later metaphases in the experiments of Table 1 also strongly support this view; the frequencies of first metaphases in the AT cultures, which are always higher even in the unirradiated samples, are notably elevated in the irradiated ones as compared to the irradiated control cultures. Thus it does not appear that the case of AT offers any real argument against the proposed central role of DNA double strand breakage in aberration formation.

Base Damage. This category of DNA lesions includes a variety of alterations frequently defined in terms of their recognition by specific endonucleases. It appears that most chemical mutagens produce lesions of this general class. Those that produce chromosomal aberrations seem to do so through local interference with the lesion-bearing polynucleotide strand's ability to serve as template for nascent strand synthesis; aberration induction is "S-dependent." Only chromatid aberrations are produced, and often, at least, only if the treated cells pass through at least part of an S phase. Chemically produced alkylations, apurinic and apyrimidinic sites, and lesions of the 5,6-dihydroxydihydrothymine type fall in this class, though little is as yet known of the specific roles played by them in chromosome aberration production.

It appears that cells from some, but not all, cases of the human recessive genetic disease Fanconi's anemia are deficient in their ability to excise lesions of the 5,6-dihydroxydihydrothymine type from their DNA [43]. As already noted, FA lymphocytes have been reported to be chromosomally sensitive to ionizing radiation administered in G_0 [19, 20], though Sasaki and Tonomura [51] were unable to confirm this. Nevertheless, we have found both lymphocytes and fibroblasts from FA patients to be abnormally sensitive to irradiation in G_2

(7). However, FA cells have also been reported to be sensitive to aberration induction by alkylating agents, including tetramethansulfonyl-d-manitol (54), nitrogen mustard and mitomycin C (51), ethylmethane sulfonate (29) and diepoxybutane (1). As will be discussed later, FA appears to be defective in DNA crosslink repair, but since some of the agents to which FA cells are reported to be sensitive are nonfunctional, FA must also be deficient in the removal of at least some simple alkylations, perhaps in an enzyme common to several DNA repair systems.

Though perhaps most often thought of as being deficient in repair of pyrimidine cyclobutane dimers, cells from patients afflicted with the excision repair forms of xeroderma pigmentosum are also sensitive to aberration production by some, but not all, alkylating agents. Those to which XP cells are sensitive are those inducing DNA repair of the "long patch" type induced by UV light (42), including N-acetoxy-2-acetylaminofluorene, polycyclic hydrocarbons and 4-nitroquinoline 1-oxide (33, 48, 56). Those to which they are not abnormally sensitive induce ionizing radiation type ("short patch") DNA repair (42), and include methyl methanesulphonate and N-methyl-N-nitro-nitrosoquanidine (48, 56). Sensitivity of XP cells to mitomycin C has also been reported (18), and since repair of DNA crosslinks appears to be normal in XP cells (50), it seems probable that the monoadducts produced by this agent produce the excess aberrations.

Ataxia telangiectasia clearly confers abnormal sensitivity to ionizing radiation, and like FA cells, AT cells are reported to be deficient in the ability to remove a form of base damage, the gamma endonuclease sensitive site, from their DNA (39). The deficiencies involved in the two diseases are different,

however, since AT cells have a normal capacity to remove the lesions of the 5,6-dehydroxydihydrothymine type (44) the repair of which is deficient in FA.

If this is the only repair deficiency in AT, the abnormal chromosomal sensitivity of G_2 AT lymphocytes to ionizing radiation implicates this form of base damage in the production of chromatid aberrations and also achromatic lesions, since the yields of both are abnormally elevated (6, 41, 57). However, AT cells are not totally unable, but rather just slow to remove gamma endonuclease sensitive base damage, and this could arise if the defect affected an intermediate step in excision. Thus it is possible that the additional aberrations are actually the consequence of repair-induced strand breaks.

A most notable cytogenetic consequence of the repair defect characterizing AT cells was discovered by Taylor, et al. (59) and is illustrated by the data in Table 1. In sharp contrast to the classical observation that irradiation of G_0 or G_1 cells (except very close to the onset of the S phase) produces only aberrations of the chromosome type, G_0 irradiation of AT lymphocytes produces chromatid aberrations as well. Though Taylor (57) has argued that an undetected strand break repair deficiency might be involved, the implication seems clear to me that the gamma endonuclease sensitive lesions, though normally repaired rapidly enough so that few if any are left when DNA synthesis begins, are capable, if present during S, of producing aberrations by precisely the same route as other forms of base damage caused by chemical mutagens.

DNA crosslinks. Bifunctional alkylating agents are capable of crosslinking a polynucleotide strand to either protein or another polynucleotide strand. Unfortunately, it is difficult to distinguish between the two, or to sort out effects caused by crosslinks from those caused by the monoadducts these agents also produce. However, Sasaki and Tonomura have quite clearly demon-

strated that FA cells are particularly sensitive to those mutagens capable of crosslinking, including mitomycin C, nitrogen mustard and treatment with 8-methoxypsoralin plus 355 nM ultraviolet light, but not notably so to the monofunctional derivative decarbamoyl mitomycin C or other monofunctional agents [49, 50, 51], thus implicating a defect in the repair of crosslinks in this disease. This has been confirmed by direct biophysical measurements on DNA from treated FA cells [15, 16]. Thus it seems certain that crosslinks do in fact give rise to chromosomal aberrations. Just how a DNA interstrand crosslink might do so is still unclear, however, since one might expect both chromatids to be affected, but some, at least, of the excess aberrations induced by crosslinking agents in FA cells appear to be types involving only one [51].

Pyrimidine Cyclobutane Dimers. These lesions are the predominant form of DNA damage induced by irradiation with ultraviolet light. As already mentioned, the existence of the photoreactivation phenomenon, which appears to be specific for removal of lesions of this type only, made it possible to be certain that virtually all of the cytogenetic consequences of ultraviolet irradiation (if not all) are produced by dimers [17]. Aberration production appeared to be entirely S dependent; in Chinese hamster and amphibian cells in culture, it was found that only chromatid aberrations resulted, and only in cells that had passed through at least part of an S phase [4, 23]. However, this may not be an entirely universal phenomenon.

Several authors have studied aberration production by ultraviolet light in normal human cells and reported that though the efficiency with which aberrations of any kind are produced appears much lower than in rodent or amphibian cells, presumably because of the much higher efficiency of the excision repair system in human cells, a few chromosome type aberrations appear in apparent

first post-irradiation mitoses (22, 34). Furthermore, Orr and Griggs (37) have also found a few chromosome type aberrations in apparent first metaphases of synchronized Chinese hamster and amphibian cell cultures given doses in excess of 20 J/M^2.

Such an exception to strict S-dependence could arise if the pre-replication excision repair of dimers occasionally resulted in creation of a double polynucleotide strand break through either coincidence of dimers close enough to each other in the two strands of a double helix to result in such a break if simultaneously excised (i.e., within an average long patch length of each other), or through occasional nuclease attack on the temporarily single stranded region of the other polynucleotide strand. With the intent of investigating this possibility, we have recently done experiments in which we irradiated separated normal human G_0 peripheral lymphocytes with 254 nM ultraviolet light and scored unequivocal first post-irradiation mitoses in cultures made with 25 μM 5-bromodeoxyuridine and differentially stained after fixation (5). However, after scoring almost a thousand cells given doses of up to 10 J/M and fixed at intervals of up to 128 culture hours (when only about 10% of the metaphases seen in 5 J/m cultures were still first divisions), we have yet to find an unequivocal chromosome type aberration in a first metaphase at any dose or time. Clearly, though the role of the pyrimidine cyclobutane dimer in S-dependent aberration production seems unequivocal, the mechanisms involved in chromosomal aberration production by ultraviolet light need further study.

The common forms of XP are deficient in pre-replication excision of ultraviolet-induced dimers, and XP cells, as already noted, are also more sensitive than cells from normal humans to chromosomal aberration induction by ultraviolet irradiation (34, 38). The so-called "variant" form of XP, on the

other hand, appears to have normal excision repair but to be defective in a post-replication repair system for ultraviolet-induced DNA damage (13, 32, 47). However, the induction of chromosomal aberrations by ultraviolet light in XP variant cells has not yet been investigated, so the role in aberration production of whatever the lesions are for which repair is deficient in such cells remains unknown. They could, since their repair is post-replicational, simply be the gaps left in nascent strands because of dimers remaining unremoved in the template strands, and the repair system itself the recombinational one we postulated to account for the production of isochromatid deletions (4).

SUMMARY

Though the roles of some specific DNA lesions in the production of chromosomal aberrations is clearly established, those of others remain unclear. While the study of aberration production in human genetic DNA repair deficiency diseases has been extremely rewarding already, eukaryotic repair systems are obviously complex, and one is tempted to feel that such studies may have raised as many questions as they have provided answers. For example, the "standard" sort of XP is chromosomally sensitive to ultraviolet light and to those chemical agents inducing ultraviolet-type DNA repair. But both it and the variant form have been reported to also be sensitive to the crosslinking agent mitomycin C in one study (18), implying a common step or steps in the repair of pyrimidine cyclobutane dimers and DNA crosslinks. However, just to complicate matters, another study of chromosomal aberration production in XP cells had found them no more sensitive to mitomycin C than normal cells (50). Similarly, FA cells, which are chromosomally sensitive to crosslinking agents, and appear to be defective in the "unhooking" of linked polynucleotide strands (15, 16, 49, 51), are reported to be chromosomally sensitive to ethylmethane sulfonate as well

(29), and to be sensitive to ionizing radiation (7, 19, 20), again implying overlapping repair systems. It seems certain that further study of chromosomal aberration production in repair deficient cells by agents inducing various DNA lesions will reveal even greater complexity in eukaryotic DNA repair systems and their role in chromosomal aberration production. Nevertheless, there seems hope, at least, that such studies may also ultimately lead to a complete understanding of the molecular mechanisms involved.

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TABLE 1. FREQUENCIES OF CHROMOSOMAL ABERRATIONS IN UNIRRADIATED AND G_0-IRRADIATED PERIPHERAL LYMPHOCYTE CULTURES FROM PATIENTS WITH ATAXIA TELANGIECTASIA AND NORMAL CONTROLS. ONLY FIRST POST-IRRADIATION MITOSES WERE SCORED. IRRADIATIONS WERE 200 R OF 250 KVP X-RAYS OR 200 R OF 60 Co GAMMA RAYS IN TWO SEPARATE EXPERIMENTS, THE RESULTS OF WHICH WERE NOT SIGNIFICANTLY DIFFERENT.

			CHROMATID TYPE(%)			CHROMOSOME TYPE(%)		
DOSE	SUBJECTS	CELLS	AL	CD	EX	DEL	RING	DIC
NONE	3 CONTROLS	461	18	6	1	0.2	0	0.2
	4 AT	289	5	8	1	5	1	4
200 R	3 CONTROLS	544	10	4	0.2	31	7	39
	4 AT	504	23	33	6	70	7	42

FIGURE LEGENDS

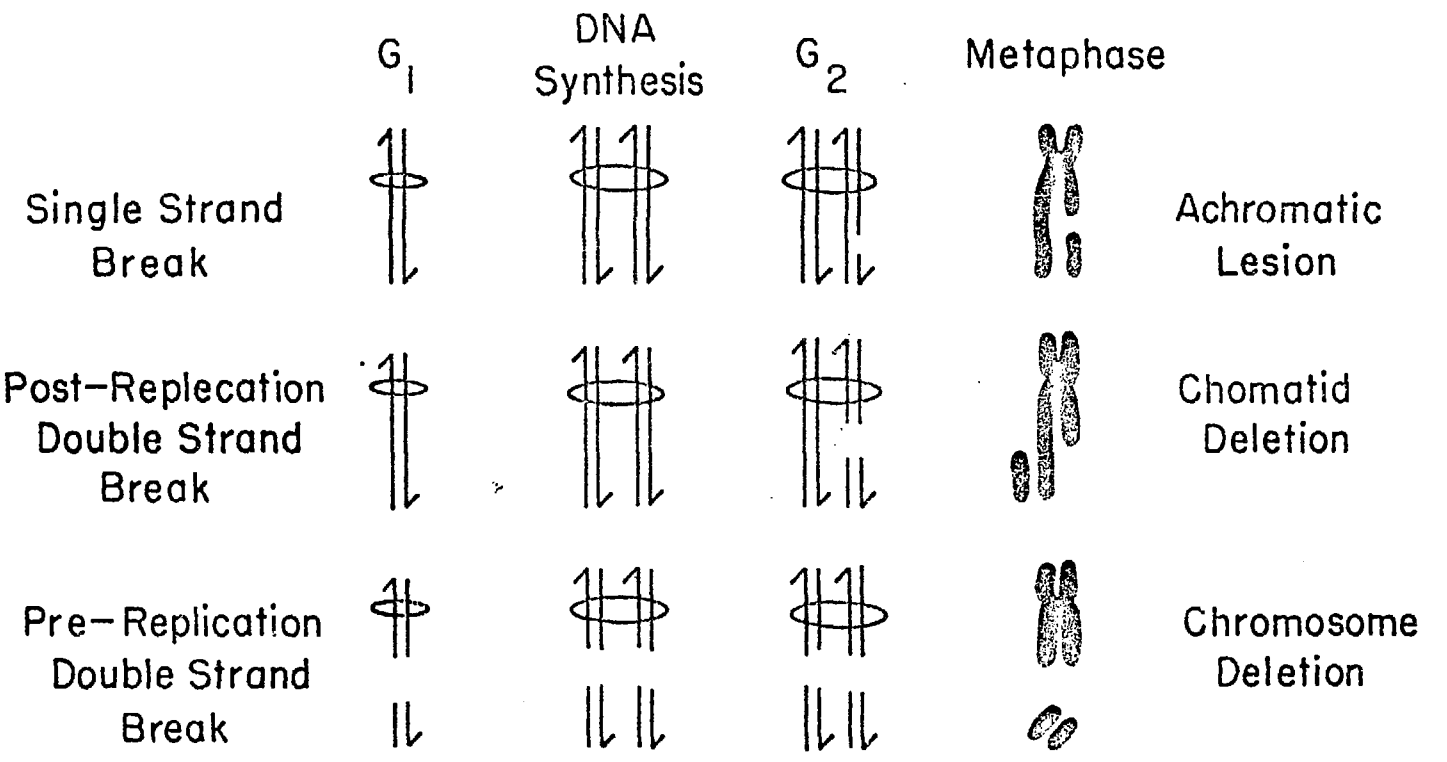
Fig. 1. Schematic representation of the roles of single and double polynucleotide strand breaks in the production of achromatic lesions and chromatid and chromosome type breaks.

Fig. 2. A possible mechanism for the origin of half-chromatid exchanges from the recombination of "double strand" breaks occurring within palindromic base sequence regions of single polynucleotide strands while paired in the "hairpin" configuration.

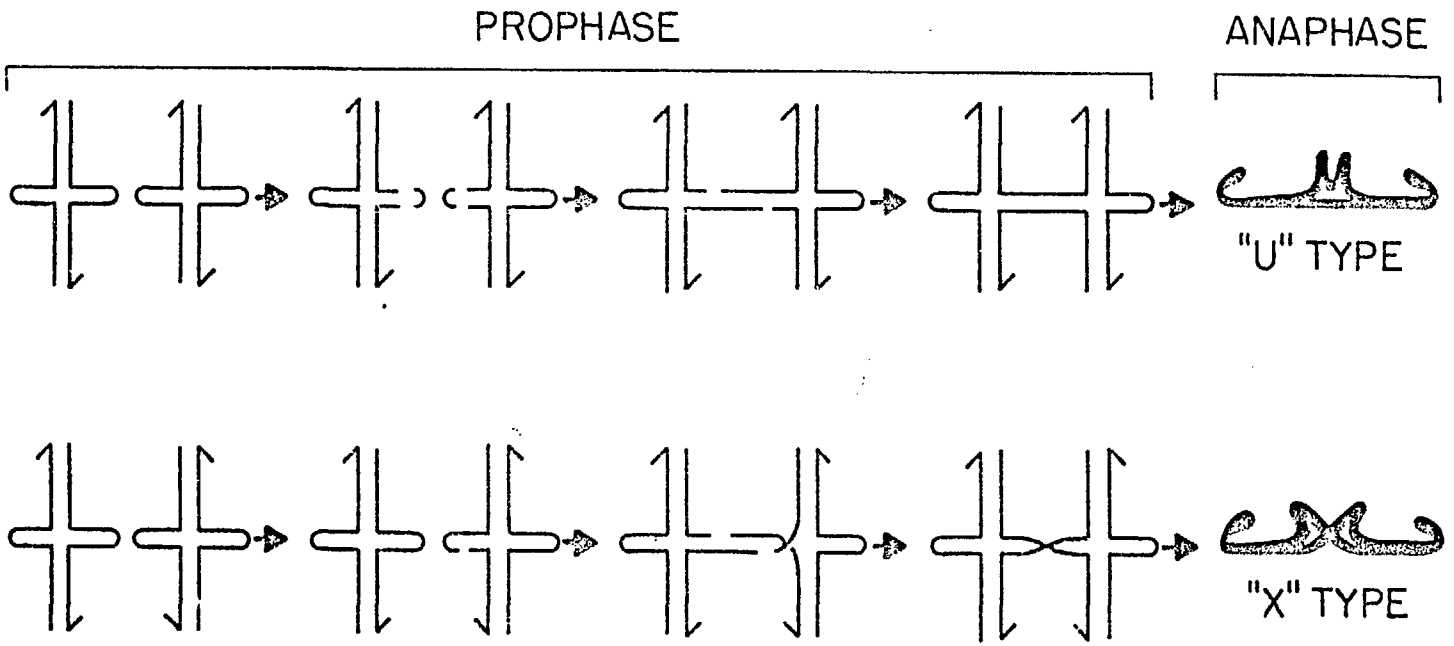
Fig. 3. Diagramatic illustration of the possible contributions of single polynucleotide strand breaks and of "base damage" to chromatid breaks through single strand nuclease (SSN) attack, normal DNA synthesis and/or endonucleolytic base damage excision (ENDON.).

Fig. 4. Diagram showing steps in proposed mechanism by which broken chromosome ends may rejoin. Ends of broken DNA double helices are rejoined through exonuclease activity, annealing of sticky ends, and repair of the remaining single polynucleotide strand breaks, just as is deliberately done in the construction of recombinant DNAs.

Fig. 5. Illustration of the way that breaks in palindromic base sequence regions of DNA could anneal to form "unavailable" break ends (telomeres?), or, in S or G_2 cells, sister unions.


G1
DNA Synthesis
G2
Metaphase
Single Strand Break
Achromatic Lesion
Post-Replecation Double Strand Break
Chomatid Deletion
Pre-Replication Double Strand Break
Chromosome Deletion

7-463-79



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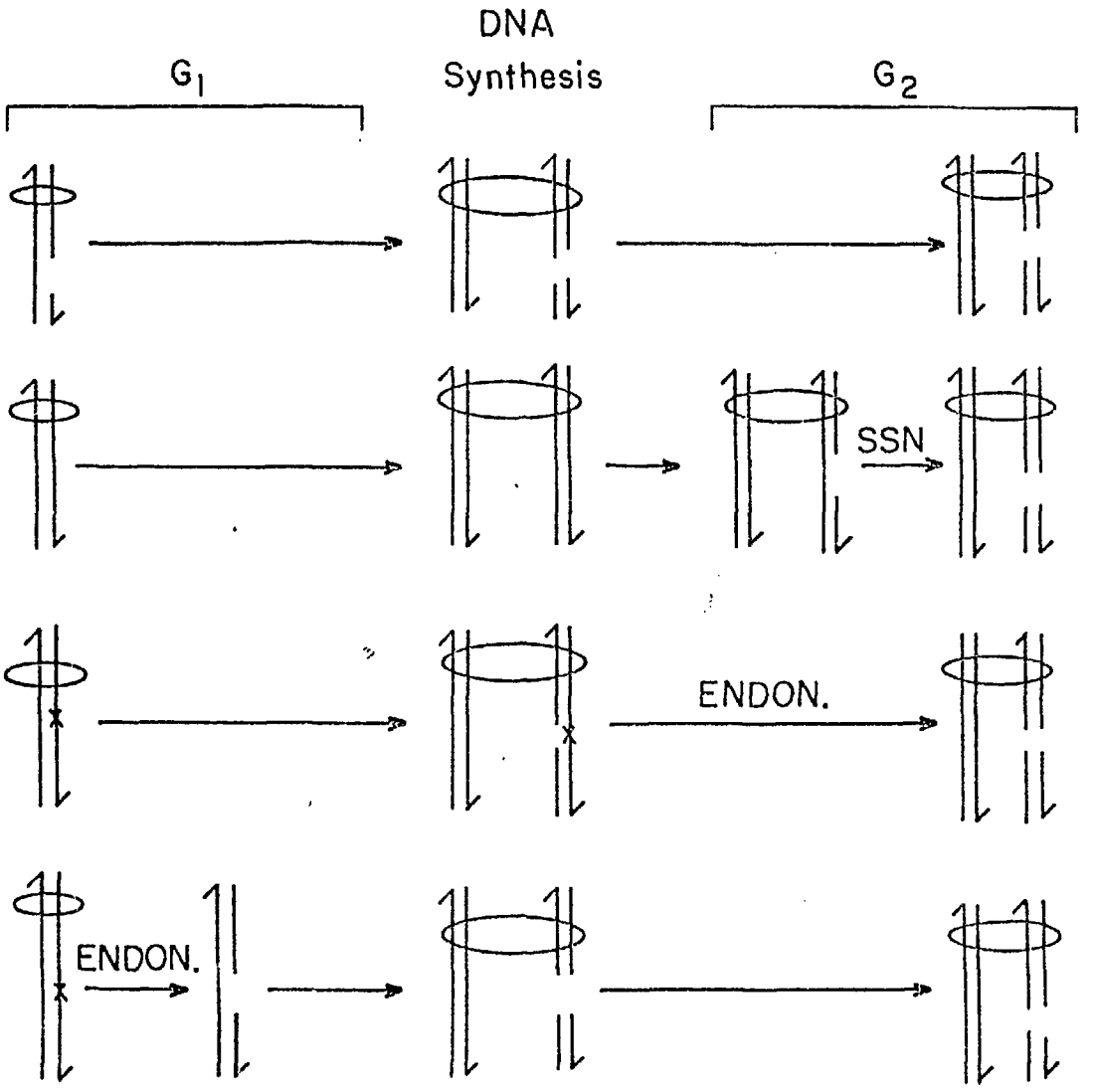

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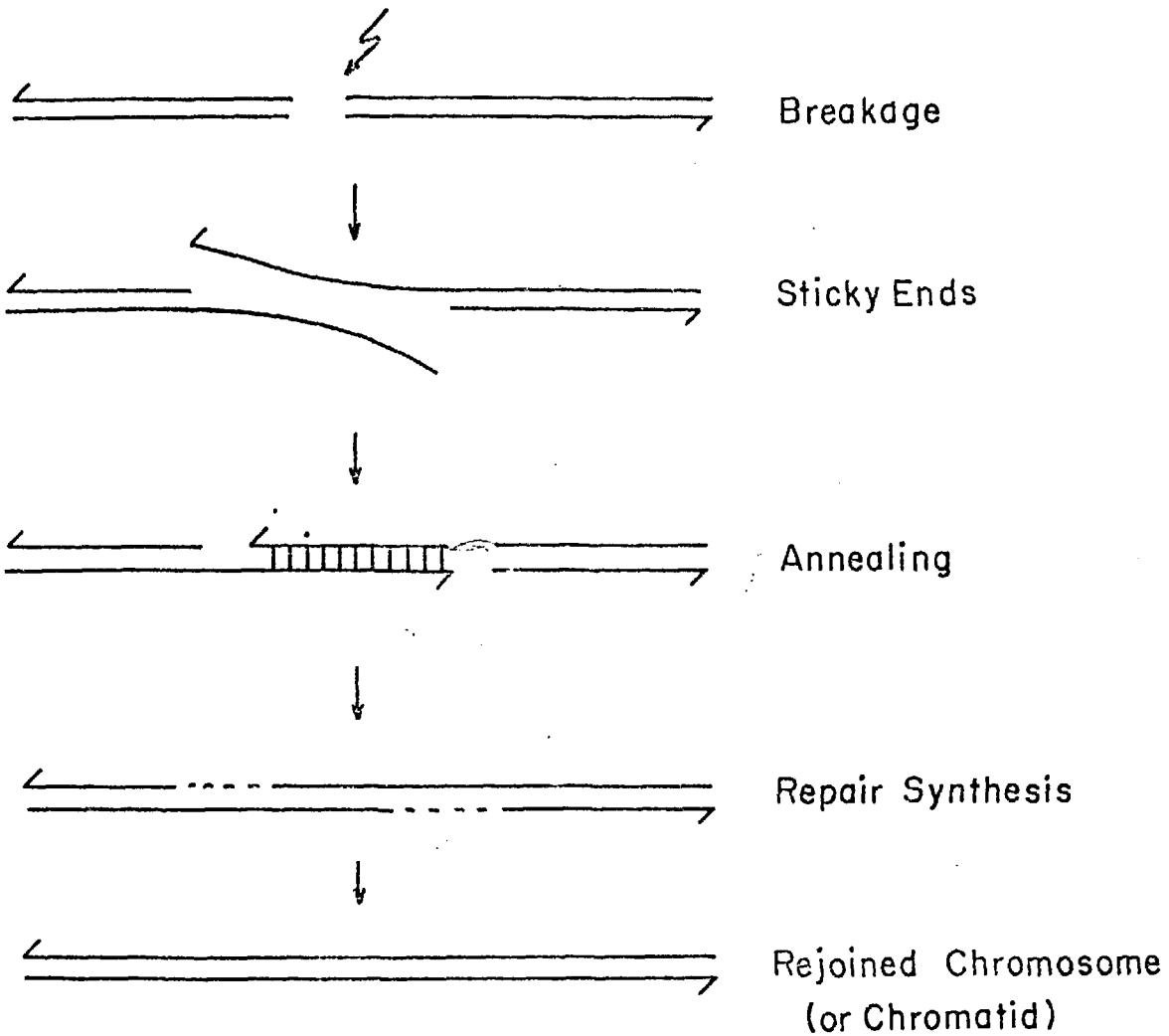
Breakage
Sticky Ends
Annealing
Repair Synthesis
Rejoined Chromosome
(or Chromatid)

7-462-79