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Biological consequences of strand breaks in plasmid and viral DNA

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Summary Some biological consequences of strand breakage in biologically active single- and double-stranded plasmid and viral DNA are examined. A double-strand break in DNA produced by restriction-endonucleases in aqueous solution is not a 100% lethal damage. The survival depends strongly on the structure of the end groups. Evidence is presented that survival is the result of a balance between degradation and repair. The enzymatically produced double-strand break (dsb) is a potentially lethal damage similar to the irradiation-produced dsb in cells.

Results with double-stranded biologically active DNA treated either with γ-rays, heat, pancrease nuclease or UV-light in aqueous solution suggest that a single-strand damage is also a potentially lethal damage. Mechanisms for conversion of single-strand damage to lethal events are discussed.

Several research groups have found an approximately one to one ratio between unrepaired DNA double strand breaks and cell deactivation under the influence of sparsely ionizing radiation (Kaplan, 1966; Ho, 1975; Krisch *et al.*, 1976; Resnick & Martin, 1976; Resnick, 1978; Frankenberg *et al.*, 1981, 1985; Hülsewede, 1985; Radford, 1985). These investigations show that this holds for variations in the experimental conditions, e.g. different gaseous conditions, different additives and under conditions of immediate or delayed plating. On this basis it was proposed that a dsb is the potentially lethal damage in irradiated cells. From a chemical point of view high-energy irradiation should generate a large variety of damaged sites in DNA. Evidence for this is the range of products found when desoxyribose or thymine is γ-irradiated in aqueous solution. The number of products is greater than 15 and 12, respectively (Schulte–Frohlinde & v. Sonntag, 1985). Obviously the results imply that most of the damaged sites in the DNA of a cell are either repaired or converted into dsb, and that enzymes are responsible for the transformation. The formation of single-strand and double-strand breaks (ssb and dsb, resp.) by the action of enzymes in cells on irradiated DNA has been reported (Bonura *et al.*, 1975; Bresler *et al.*, 1979; Bryant, 1986).

The view gives rise to various questions. Among them are the following: Firstly, is a dsb really the main damage leading to deactivation or are other kinds of damages also lethal? Secondly, are all dsb alike? In other words does the cell recognize differences between dsb with different end groups? Thirdly, is deactivation of the DNA by high-energy irradiation due to an immediate radical-induced loss of genetic information or due to a competition between repair and either fixation or degradation? In connection with this, does deactivation of double-stranded DNA caused by a damage on only one strand (a single-strand damage, SSD) occur? Answers to these questions which have been given by various research groups will be discussed in the present paper. The system studied is biologically active plasmid or viral DNA treated in aqueous solution. The biological activity is expressed in *E. coli* strains.

Survival of biologically active plasmid DNA containing one double-strand break

In order to obtain insight into the problems raised, one has to design experiments which are as unequivocal as possible, which is difficult since our knowledge concerning the damage in DNA induced by high-energy irradiation is incomplete and so is our knowledge of the various enzymatic repair systems of the cells.

The experiment of choice is to measure the survival of biologically active DNA molecules all of which carry exactly the same damage. The advantage of such an experiment is that no statistics is involved in the interpretation of the results. This experiment has been carried out by various research groups in the following way: Biologically active circular double-stranded plasmid DNA (pBR 322) has been treated with endonucleases, which introduce only one dsb into the circular DNA (supercoiled DNA, scDNA) thereby linearizing the DNA. With this linearized DNA (linDNA) *E. coli* (in our case *E. coli* strain SFX, rec A^+, rec BC^-, rec F^+, sbcB) are transformed and the survival of the DNA is measured. The pBR 322 plasmid contains besides other genes the tet^R and the amp^R gene and the origin of replication.

Table I Survival of circular double-stranded plasmid DNA (pBR 322) in *E. coli* SFX (rec A^+) linearized by enzymatic action (Bien & Schulte-Frohlinde, 1986)

Enzyme	*Structure of the ends*	*Survival in % of SC form*
BAM HI	Sticky end	2.5 %
PVU II	Blunt end	0.11%

A typical outcome of these kinds of experiments is shown in Table I. A double-strand break is not absolutely lethal. The survival depends on the structure of the end group. The survival of a linDNA with overlapping ends generated with Bam HI is more than 10 times better than that of a DNA with a dsb with blunt ends (Bam HI cuts the pl DNA pBR 322 in the tet^R-gene producing dsb with an overlapping end of four base pairs and Pvu II generates a dsb with blunt ends in the region between the tet^R-gene and the origin of replication). Similar results have been obtained with other restriction-endonucleases (Sal I and Hpa I) and other *E. coli* strains (*E. coli* SK 1592, C 600, AB 1157) (Thompson & Achtman, 1978; Garaev *et al.*, 1982; Conley & Saunders, 1984). Since reproduction and therefore survival is only possible for circular DNA, the dsb of the linDNA has to be rejoined. This occurs enzymatically. The survival is the result of enzymatic repair. The repair occurs without the help of general homologous recombination. Homologous recombination is not possible in the case presented (Table I) since no homologous intact genes are available. It has to be assumed that the repair occurs with the help of a ligase in addition to other enzymes because a strain with ligase overproduction exhibits a threefold better survival for linearized DNA. The rec A repair system is not strongly involved because a rec A^- strain does not show lower survival and UV-induction of SOS repair in a rec A^+ strain is only small (factor less than 2) (Bien & Schulte-Frohlinde, 1986).

What do the results in Table I tell us?

The formation of the linDNA by the treatment with endonucleases does not destroy the genetic information. The chemical identity and the sequence of the bases is completely retained in the linDNA. The only damage is the breaking of two phosphoric acid ester bonds in the two complementary strands. The surprisingly low survival of the linDNA (Table I) therefore is not due to a loss of genetic information prior to the probe for survival.

Before we can answer the question as to what else determines the survival of linDNA in a cell, the fate of the non-surviving fraction of the DNA should be discussed. The fate of the linDNA is assumed to be degradation by exonucleases. Evidence for this is the appearance of mutants which carry circular DNA with a molecular weight smaller than the original (i.e. they carry deletions). For linDNA with overlapping ends the percentage of mutants with deletions is 1%. For linDNA with blunt ends the number of mutants carrying deletions is over 40% (Bien, 1986). Furthermore the influence of the structure of the end groups of the linDNA and that of the presence of ligase in the cells on survival indicates that degradation of the linDNA *versus* repair determines the survival of the DNA. These results suggest that repair by rejoining and enzymatic degradation are competing processes. Obviously survival of a linDNA is a rare event in *E. coli* cells and degradation is the rule.

The results mean that a dynamic relationship exists between damage and survival. A damaged site in the DNA may first have the chance to encounter repair enzymes and the damage will then be repaired, or the damaged site first encounters an exonuclease or another unfavorable enzyme and the DNA will be degraded before repair occurs. This dynamic situation then leads to the result that a portion of the linDNA molecules survives, whereas another portion will be deactivated. Expressed on an operational basis one would say that the enzymatically produced dsb has the property of a potentially lethal damage (PLD). Does the result that the survival of enzymatically damaged DNA is the outcome of a balance between repair and degradation also hold for irradiated DNA? This will be discussed for biologically active single- and double-stranded DNA irradiated in aqueous solution.

Single-stranded biologically active DNA irradiated in aqueous solution

The first example is that of biologically active ssDNA γ-irradiated in aqueous solutions. The experimental results are summarized in Table II.

A single-strand break (ssb) produced by an enzyme is practically 100% lethal. The linearized ssDNA is degraded before repair has taken place, although repair should be possible. The case of a missing base e.g. ssDNA with an apurinic site (AP site) is of interest because here the interpretation is possible that the 100% lethality is the result of loss of genetic information. A closer inspection, however, reveals that in principle this need not be the case because an enzymatic replacement of the missing base could lead to a 25% survival if the replacement occurs statistically. The case of the thymine glycol moiety is an example of a potentially lethal damage in ssDNA which is neither completely lethal nor harmless. Of particular interest is biologically active dsDNA irradiated in aqueous solution.

Table II Lethality of damaged biologically active single-stranded DNA (ϕX174)

Damage	*Lethality*
SSB	100%
Apurinic site	100%
Thymine glykol	30%

Results: for ssb see Dertinger and Jung (1969); for AP sites from Lafleur (1978), Lafleur *et al.* (1981); for thymine glykol from Cerutti (1976).

Double-stranded biologically active DNA irradiated in aqueous solution

For dsDNA the situation is complicated by the fact that damage in one strand does not destroy the information available. If a damage is generated in only one strand the integrity of the dsDNA can be restored by the enzymatic repair system in the cells with the help of the information available from the complementary strand. Indeed, enzymatic repair of ssb occurs within a few minutes mainly by an excision-resynthesis repair and to a smaller extent by other repair mechanisms. On this basis damage in only one strand should not lead to deactivation of biologically active dsDNA.

Table III Lethality of damaged biologically active double-stranded DNA

DNA	*Damage*	*Lethality*	*Dependence on dose*
PBR 322	ssb + base damage γ-irradiation	$<5\%$	1. order
ϕX174	Apurinic site heat treatment	$6\% \pm 3$	1. order
ϕX174	ssb pancrease nuclease	$5\% \pm 2$	1. order
PBR 322	1 DSB endonucleases	$>95\%$	

Results: for ssb + base damage from **Figure 1**; for AP sites from Lafleur *et al.* (1981) and Lafleur and Loman (1986); for ssb by pancrease nuclease from Jansz *et al.* (1968); for dsb by endonucleases see **Table I**.

This leads to the expectation that only an event which includes both strands creates a lethal damage. If this is true then γ-irradiation of biologically active dsDNA in aqueous solution should lead to deactivation in a second order dose dependence as long as two radicals are necessary to create two damaged sites at the two complementary strands. The expected result is not obtained however (van der Schans *et al.*, 1973). With *E. coli* cells as hosts, in absence of general homologous recombination, linear dose dependencies have been observed for more than three decades when ΦX174 dsDNA is irradiated in aqueous solution (Blok & Loman, 1986). Linear survival curves are interpreted as single hit–single target curves on the basis of the target theory. Several explanations have been advanced to account for this result.

One possibility is that spur effects may be responsible. In spurs more than one radical is generated. This allows the possibility that both strands may be damaged with one hit. This attractive hypothesis which was put forward by Ward (1981) is useful to explain the damage of DNA in cells where the DNA is surrounded by a high concentration of organic material which scavenges most radicals, such that the contribution of the indirect effect to deactivation is low. However in N_2O saturated aqueous solutions with relatively low scavenger concentrations, spur reactions cannot contribute to the damage of the DNA.

Another idea is that a single OH radical is able to damage both strands. Using supercoiled phage DNA and gel chromatography Blok and Loman (1986) have found, that γ-irradiation of the dsDNA in aqueous solution with low doses leads to the formation of dsb in a first order dependence on dose. A linear-quadratic dose dependence for

the induction of dsb was found by Hagen (1967) and by various other research groups (Freifelder & Trumbo, 1969; van der Schans *et al.*, 1973) for dsDNA γ-irradiated in aqueous solution. Using calf thymus dsDNA in N_2O saturated aqueous solution and light scattering as an analytical tool (Siddiqi & Bothe, 1986) observed, at low doses, a first order formation of dsb with a G value of 0.035 on which, with increasing doses, the expected second order formation of dsb is superimposed. The $G_{(dsb)}$ values for initiation by one OH radical in N_2O saturated solutions in the absence and in the presence of oxygen are very similar ($G_{(dsb)}=0.035$ and 0.032, respectively).

The interpretation of these observations is that a single OH radical is capable of producing a dsb. The mechanism is very probably radical transfer from one chain to the other (interstrand H-transfer). At first the DNA radical produced by the OH radical will generate a ssb. During this process a sugar radical will be formed which has a long lifetime because it disappears only by a bimolecular reaction with another radical. Since the lifetimes of the DNA peroxyl radicals are in the range of hundreds of milliseconds (Schulte-Frohlinde *et al.*, 1986) ample time is available for a radical transfer to the second strand even in a reaction without a change in free energy. The transferred radical will then lead to sb formation, so that in sum a dsb has occurred.

The question is whether or not the yield of this kind of double strand damage (DSD) caused by a single OH radical is so large that the first order survival curve in aqueous solution can be explained. The answer is no, as long as a dsb as the lethal event is involved because the measured G value of 0.035 is too low to explain the deactivation. The deactivation of φX174 ssDNA by OH radicals has an efficiency of $\approx$20% (Blok & Loman, 1986) which leads to a G value for deactivation by OH radicals reacting with DNA in air of $\approx$0.6. Allowing for a 4 fold higher radiation resistance of dsDNA leads to G=0.12 or G$\approx$0.24 in N_2O/O_2. There is the possibility that in addition to the formation of a dsb by a single OH radical two sugars or two bases or a sugar and a base in opposite strands in close proximity may be damaged by one OH radical without the formation of strand breaks. Although the yield for this process is expected to be only slightly larger than that for a dsb, this possibility is not ruled out experimentally.

The question now arises whether only a DSD is responsible for deactivation of a dsDNA irradiated in aqueous solution, since there are several pieces of evidence in favour of a single-strand damage (SSD) as a potentially lethal damage. Jansz and Pouwels (1965) observed with pancreas nuclease which produces ssb randomly in a φX174 dsDNA that the number of ssb increased linearly with time and survival decreased mono-exponentially with time over three orders of magnitude. A fast initial period observed was assigned to the conversion from the supercoiled to the relaxed form. For this process only one ssb is necessary (Jansz *et al.*, 1968). From the slope of the mono-exponential decay of the biological activity it followed that 1 ssb out of 20 leads to deactivation. If it is assumed that the nuclease used does nothing else but produce ssb by hydrolysis of phosphoric acid ester bonds, then a ssb may be potentially lethal with a probability of $<5\%$ (Table III). Other research groups found essentially similar results (van der Schans *et al.*, 1973).

This conclusion is supported by a further piece of evidence. Lafleur and Loman (1986) have shown that the decay of the biological activity of φX174 dsDNA by a heat treatment producing apurinic sites results in a mono-exponential survival curve. Although the decay had been followed over only a small range it was possible to demonstrate that 17 ($\pm$6) apurinic sites are necessary to generate one deactivation (Table III). Since the survival curve is mono-exponential it has to be concluded that only one AP site produced is responsible for deactivation. The other 16 AP sites have nothing to do with the deactivation.

From the heat treatment as well as from the nuclease treatment no formation of a double-strand damage (no DSD) is expected and indeed not found (Lafleur and Loman, 1986).

This leads to the assumption that a single-strand damage (SSD) may be a potentially lethal damage.

A further hint in the direction of this interpretation can be seen in Figure 1, where the survival of plasmid dsDNA as a function of the γ-irradiation dose is compared with the formation of dsb, ssb and that of an assumed base damage. The base damage has been proposed to be roughly 3–10 times more frequent than ssb. Ward (1985) gives a figure of 3.5. For more details concerning base damage see Wallace (1983).

Figure 1 shows that the survival does not correlate with dsb because the yield of dsb is too low. But there is also no correlation with ssb or base damage. Their yield is too large. Analogous results have been obtained by Taylor and Ginoza (1967) for φX174 dsDNA. This result and the absence of a correlation suggests that base damage and ssb may be regarded as a potentially lethal damage, which means that they lead to deactivation with a low yield. This and other results indicate that every kind of lesion in biologically active DNA is potentially lethal (Schulte-Frohlinde, 1986).

An example which convincingly shows a SSD as a potentially lethal damage is the pyrimidine dimer produced by UV light in biologically active dsDNA. The pyrimidine dimer is an intrastrand damage (an SSD) and the UV-illumination of φX 174 dsDNA gives a mono-exponential survival curve over 5 decades (Yarus & Sinsheimer, 1964). Since a pyrimidine dimer is reparable it is clear that it is a potentially lethal damage and that the formation of a single one of the pyrimidine dimers formed represents the lethal damage.

The suggestion that a SSD may be potentially lethal in dsDNA leads to further questions. Is there a chance that in a cell a SSD is transformed into a dsb? Bender *et al.* (1974) assume that a dsb is produced from a ssb by a single-strand nuclease in cells or produced at the replication fork. Another hypothesis is that the damage brought about by a SSD produces solely a functional damage but not a replicational one. This is possible because the information for the phenotype function is localized on one strand only whereas either strand can serve for replication. If it happens that an essential phenotype function cannot be carried out due to

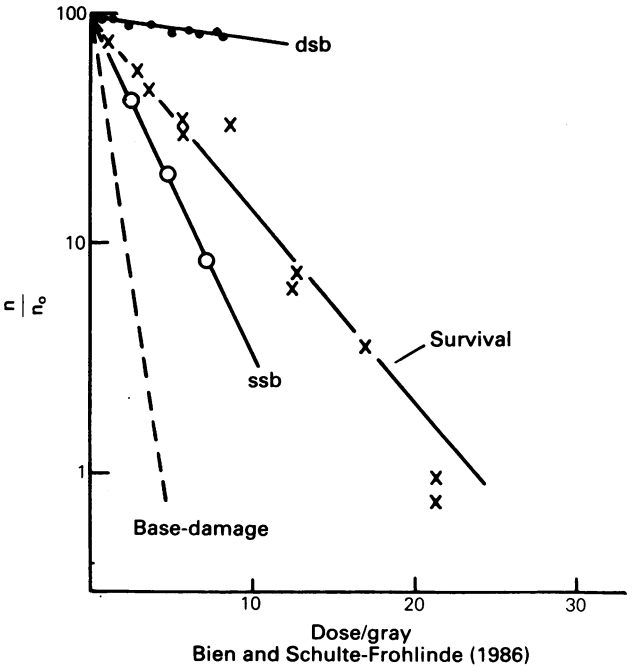

Figure 1 DNA damage and survival. Irradiation in aqueous solution in air, plasmid dsDNA pBR 322 expressed in *E. coli* K-12 SFX (Bien & Schulte-Frohlinde, 1986).

SSD formation then replication may be inhibited (see Harm, 1980). This necessarily requires the destruction of certain genes at the genome and leads to the assumption that there exists a sensitive site in the DNA, the size of which should be amenable to correlation with the efficiency of deactivation under certain conditions. However, this explanation is restricted to certain phages and may be not generally applicable (Dertinger & Jung, 1969). Another possibility might be that to a certain extent an attempt of long-patch repair may convert a SSD into a DSD by cutting out such a long piece of ssDNA from the dsDNA (more than 1500 base pairs by definition, Cooper, 1982) that an overlap with a SSD in the complementary strand occurs. However, it is also possible that the conversion of a SSD into a lethal damage is simply the work of nucleases which degrade one strand of the dsDNA so efficiently that overlapping with a SSD in the complementary strand occurs in every case. This seems possible because on the average approximately 20 SSD have to be present in the plasmid dsDNA before 1 lethal event occurs (Table III). In other words one degradation takes place for $\approx$19 DSD repaired. In this case the mono-exponential survival curve represents a pseudo-first order survival curve with respect to the number of damaged sites which have to act together in order to produce the lethal damage. However, it is still an open question whether or not degradation of DNA starting at damaged sites plays the main role in the deactivation of γ-irradiated DNA since the formation of mutants carrying deletions is very small (0.1%, Bien, 1986). Other enzymatic mechanisms may contribute.

Comparison with the deactivation of cells

In cells the concentration of organic material other than DNA is so high that the indirect effect plays a much smaller role than in aqueous solution. This has two consequences. Firstly, spur effects become important even with sparsely ionizing radiation, as Ward (1981) has pointed out. Hagen and coworkers (Andrews *et al.*, 1984; Martin-Bertram, 1983) have found experimental evidence for the locally multi-damaged sites postulated in the DNA of γ-irradiated cells. Secondly, although the direct effect leads in principle to similar products as the indirect effect, the distribution of the products is different and so are the rates for ssb and product formation. In selected cases (e.g. polyuridylic acid) the rate for strand break formation has been found to be the same for OH and for laser-induced ssb-formation (Schulte-Frohlinde *et al.*, 1985). In other cases, especially with DNA, different rates are observed (Opitz & Schulte-Frohlinde, 1986).

It is an open question under which circumstances a comparison of cell deactivation with the deactivation of DNA irradiated in aqueous solution leads to meaningful results. A comparison of some experimental results reveals similarities. First of all, the deactivation of cells without repair is of first order with respect to dose in the majority of cases. This is analogous to the results found for the deactivation of plasmid and viral DNA irradiated in aqueous solution.

In this connection it is of interest that Fielden and coworkers (Fielden *et al.*, 1978) with their rapid lysis techniques observed a linear relationship between the formation of single-strand breaks in the genome of irradiated *E. coli* cells and deactivation of the cells as a function of dose ($\approx$60 ssb per one lethal hit including alkaline labile sites). A relationship of 1:$\approx$20 for the ratio of lethal hits to non-rejoining strand breaks determined on alkaline sucrose gradient has been found by Goodhead *et al.* (1978) for mammalian cells. Ritter *et al.* (1978) found a ratio of 1:50. This points to a closer relationship between the results of irradiation of plasmid or viral DNA in aqueous solution and those obtained with cells than hitherto expected. Also irradiation of ds φX174 DNA, accumulated and attached inside *E. coli* cells gave comparable results as irradiation of this DNA in aqueous solution (Blok & Loman, 1986). This supports again the observed similarities, given in this section, between irradiation of DNA in aqueous solutions and in cells. A further element of similarity is the observed drastic degradation of DNA following irradiation of *E. coli* cells (results summarized by Ginoza, 1967). The results indicate that analogous to the degradation of enzymatically linearized DNA in *E. coli* cells, DNA irradiated in cells are degraded by nucleases. Furthermore, Bryant (1985) has shown that permeabilized Chinese hamster cells treated with the restriction endonuclease Pvu II produces mutants carrying deletion and chromosomal aberrations. These similarities do not allow the conclusion that single-strand damages are primarily responsible for the deactivation of DNA irradiated in a cell. However, this possibility does not seem to be excluded for conditions of low repair.

General conclusions

In this article various questions have been discussed concerning the survival of damaged plasmid or viral DNA in *E. coli* strains. The results indicate that for the enzymatically linearized DNA (DNA containing one dsb) the mechanism for deactivation is a degradation by nucleases and survival is the result of a balance between degradation and enzymatic repair. The deactivation observed is not due to a loss of genetic information, because in the enzymatically linearized DNA the sequence and the identity of the bases is not changed.

From the properties of the biologically active DNA subjected to heat, pancrease nuclease (nuclease I), UV-light and γ-rays in aqueous solution it follows that in ss and in dsDNA *every* kind of damage including a single-strand damage is potentially lethal.

Whether or not a certain kind of damage will be repaired, degraded or undergoes fixation and with which rate depends on the presence of suitable nucleases and repair enzymes, which is genetically controlled (for an influence of the genotype on the repair of differently damaged DNA see Nabben *et al.*, 1984). From the first order formation of the various lesions with respect to dose it follows that it is a single event, which leads to deactivation, even in the case of the formation of a single-strand damage. Various possible explanations for this result and possible consequences for the deactivation of cells have been discussed.

At last the question may be posed whether or not certain kinds of damage may exist which are so difficult to repair that they can be considered as lethal *per se*. At present there is no indication that such a damage plays an important role in deactivation of dsDNA γ-irradiated in aqueous solution as long as the host cells have a fully developed repair system. The reason is that the number of potentially lethal lesions (e.g. base damage and ssb) which lead to deactivation is so large that a possible contribution of a non-reparable 100% lethal lesion has no chance to be detected. This is probably due to the limited time available for repair in *E. coli* strains which cannot be made long enough for the repair of every potentially reparable lethal damage.

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References

ANDREWS, J., MARTIN-BERTRAM, H. & HAGEN, U. (1984). S1 Nuclease-sensitive sites in yeast DNA: an assay for radiation-induced base damage. *Int. J. Radiat. Biol.*, **45**, 497.

BENDER, M.A., GRIGGS, H.G. & BEDFORD, J.S. (1974). Mechanisms of chromosomal aberration production III. Chemicals and ionizing radiation. *Mutation Res.*, **23**, 197.

BIEN, M. & SCHULTE-FROHLINDE, D. (1986). Comparison of the activity of biologically active DNA damaged by irradiation or by restriction endonucleases. (In preparation).

BIEN, M. (1985). In Plasmid-DNA erzeugte ^{60}Co-Gammastrahlenschäden und ihre Reparatur in *E. coli* mit und ohne SOS-Induktion. Ein Vergleich mit enzymatisch linearisierter DNA. *Dissertation: Ruhr-Universität Bochum.*

BLOK, J. & LOMAN, H. (1986). Bacteriophage DNA as a model for correlation of radical damage to DNA and biological effects. In *Mechanisms of DNA Damage and Repair,* Simic, M.G. *et al.* (eds) p. 75. Plenum Press: New York.

BONURA, T., SMITH, K.C. & KAPLAN, H.S. (1975). Enzymatic induction of double-strand breaks in γ-irradiated *Escherichia coli* K_{12}. *Proc. Natl Acad. Sci. USA,* **72,** 4265.

BRESLER, S.E., NOSKIN, L.A., KUZOLEVA, N.A. & NOSKINA, I.G. (1979). The nature of the damage of *E. coli* DNA induced by γ-radiation. *Int. J. Radiat. Biol.,* **36,** 289.

BRYANT, P.E. (1985). Enzymatic restriction of mammalian cell DNA: evidence for double-strand breaks as potentially lethal lesions. *Int. J. Radiat. Biol.,* **48,** 55.

BRYANT, P.E. (1986). Immediate and repair induced DNA double-strand breaks in mammalian cells. In *Mechanisms of DNA Damage and Repair,* Simic, M.G. *et al.* (eds) p. 171. Plenum Press: New York.

CERUTTI, P.A. (1976). Base damage induced by ionizing radiation. In *Photochemistry and Photobiology of Nucleic Acids,* Wang, S.Y. (ed) Vol. 2, p. 375. Academic Press: New York.

CONLEY, E.C. & SAUNDERS, J.R. (1984). Recombination-dependent recircularization of linearized pBR 322 plasmid DNA following transformation of *Escherichia coli. Mol. Gen. Genet.,* **194,** 211.

COOPER, P.K. (1982). Characterization of long patch excision repair of DNA in ultraviolet-irradiated *E. coli*: an inducible function under rec-lex control. *Mol. Gen. Genet.,* **185,** 189.

DERTINGER, H. & JUNG, H. (1969). *Molekulare Strahlenbiologie.* Springer: Berlin.

FIELDEN, E.M., SAPORA, O. & LOVEROCK, P.S. (1978). The relation between survival and the initial yield of DNA strand breaks for *E. coli* B/r. *Proc. Sixth Symposium on Microdosimetry,* Booz, J. & Ebert, H.G. (eds) p. 905. EUR 6064: Brussels, Belgium.

FRANKENBERG, D., FRANKENBERG-SCHWAGER, M., BLÖCHER, D. & HARBICH, R. (1981). Evidence for double-strand breaks as the critical lesions in yeast cells irradiated with sparsely or densely ionizing radiation under oxic or anoxic conditions. *Radiat. Res.,* **88,** 524.

FREIFELDER, D. & TRUMBO, B. (1969). Matching of single-strand breaks to form double-strand breaks in DNA. *Biopolymers,* **7,** 681.

GARAEV, M.M., BOBKOV, A.F., BOBKOVA, A.F., KALININ, V.N., SMIRNOV, V.D., KHUDAKOV, Y.E. & TIKCHONENKO, T.I. (1982). The site-specific deletion in plasmid pBR 322. *Gene,* **18,** 21.

GINOZA, W. (1967). The effects of ionizing radiation on nucleic acids of bacteriophages and bacterial cells. *Ann. Rev. Microbiol.,* **21,** 325.

GOODHEAD, D.T., THACKER, J. & COX, R. (1978). Non-rejoining DNA breaks and cell inactivation. *Nature,* **272,** 379.

HAGEN, U. (1967). Bestimmung von Einzel- und Doppelbrüchen in bestrahlter Desoxyribonukleinsäure durch Molekulargewichtsverteilung. *Biochim. Biophys. Acta,* **134,** 45.

HARM, W. (1980). *Biological Effects of Ultraviolet Radiation.* p. 71. University Press: Cambridge.

HO, K.S.Y. (1975). Induction of DNA double-strand breaks by X-rays in a radiosensitive strain of yeast Saccharomyces cerevisiae. *Mutation Res.,* **30,** 327.

HÜLSEWEDE, J.W. (1985). *Doppelstrangbrüche in der DNA in vivo und in vitro nach ^{60}Co-γ-Bestrahlung.* Dissertation: Ruhr-Universität Bochum.

JANSZ, H.S. & POUWELS, P.H. (1965). Structure of the replicative form of bacteriophage φX174. *Biochem. Biophys. Res. Commun.,* **18,** 589.

JANSZ, H.S., BAAS, P.D., POUWELS, P.H., VAN BRUGGEN, E.F.J. & OLDENZIEL, H. (1968). Structure of replicative form of bacteriophage φX174. V. Interconversions between twisted, extended and randomly coiled forms of cyclic DNA. *J. Mol. Biol.,* **32,** 159.

KAPLAN, H.S. (1966). DNA strand scission and loss of viability after X-irradiation of normal and sensitized bacterial cells. *Proc. Natl Acad. Sci. USA,* **55,** 1442.

KRISCH, R.E., KRASIN, F. & SAURI, C.J. (1976). DNA breakage, repair and lethality after ^{125}I decay in rec$^+$ and rec A strain of *Escherichia coli. Int. J. Radiat. Biol.,* **29,** 37.

LAFLEUR, M.V.M. (1978). *Radiation damage in DNA: significance of alkali-labile sites.* Dissertation: Vrije Universiteit: Amsterdam.

LAFLEUR, M.V.M. & LOMAN, H. (1986). Personal Communication.

LAFLEUR, M.V.M., WOLDHUIS, J. & LOMAN, H. (1981). Some characteristics of apurinic sites in single- and double-stranded biologically active φX174 DNA. *Nucleic Acid Res.,* **9,** 6591.

MARTIN-BERTRAM, H., RUMPF, E. & WINKLER, C. (1983). Evidence for radiation-induced bulky lesions in DNA. *Radiat. Environ. Biophys.,* **21,** 305.

NABBEN, F.J., LAFLEUR, M.V.M., SIKKERS, J.C.M., LOMAN, A.C., RETEL, J. & LOMAN, H. (1984). Repair of damage in double-stranded φX174 (RF) DNA due to radiation-induced water radicals. *Int. J. Radiat. Biol.,* **45,** 379.

OPTIZ, J. & SCHULTE-FROHLINDE, D. (In preparation).

RADFORD, I.R. (1985). The level of induced DNA double-strand breakage correlates with cell killing after X-irradiation. *Int. J. Radiat. Biol.,* **48,** 45.

RESNICK, M.A. & MARTIN, P. (1976). The repair of double-strand breaks in the nuclear DNA of Saccharomyces cerevisiae and its genetic control. *Mol. Gen. Genet.,* **143,** 119.

RESNICK, M.A. (1978). Similar responses to ionizing-radiation of fungal and vertebrate cells and the importance of DNA double-strand breaks. *J. Theor. Biol.,* **71,** 339.

RITTER, M.A., CLEAVER, J.E. & TOBIAS, C.A. (1978). Non-rejoining DNA breaks and cell inactivation. *Nature,* **272,** 380.

SCHANS, G.P. VAN DER, BLEICHRODT, J.F. & BLOK, J. (1973). Contribution of various types of damage to inactivation of a biologically-active double-stranded circular DNA by gamma radiation. *Int. J. Radiat. Biol.,* **23,** 133.

SCHULTE-FROHLINDE, D. (1986). Comparison of mechanisms for DNA strand break formation by the direct and indirect effect of radiation. In *Mechanisms of DNA Damage and Repair,* Simic, G.M. *et al.* (eds) p. 19. Plenum Press: New York.

SCHULTE-FROHLINDE, D. & SONNTAG VON, C. (1985). Radiolysis of DNA and model systems in the presence of oxygen. In *Oxidative Stress,* Sies, H. (ed) p. 11. Academic Press: London.

SCHULTE-FROHLINDE, D., BEHRENS, G. & ÖNAL, A. (1986). Lifetime of peroxyl radicals of poly(U), poly(A) and single- and double-stranded DNA and the rate of their reaction with thiols. *Int. J. Radiat. Biol.,* **50,** 103.

SCHULTE-FROHLINDE, D., OPITZ, J., GÖRNER, H. & BOTHE, E. (1985). Model studies for the direct effect of high-energy irradiation on DNA. Mechanism of strand break formation induced by laser photoionization of poly U in aqueous solution. *Int. J. Radiat. Biol.,* **48,** 397.

SIDDIQI, M. & BOTHE, E. (1986). Single- and double-strand break formation in DNA irradiated in aqueous solutions. Dependence on dose and OH radical scavenger concentration. (In preparation).

TAYLOR, W.D. & GINOZA, W. (1967). Correlation of γ-ray inactivation and strand scisson in the replicative form of φX174 bacteriophage DNA. *Proc. Natl Acad. Sci. USA,* **58,** 1753.

THOMPSON, R. & ACHTMAN, M. (1979). The control region of F sex factor DNA transfer cistrons: physical mapping by deletion analysis. *Mol. Gen. Genet.,* **169,** 49.

WALLACE, S.S. (1983). Detection and repair of DNA base damages produced by ionizing radiation. *Environ. Mutagenesis,* **5,** 769.

WARD, J.F. (1981). Some biochemical consequences of the spatial distribution of ionizing radiation-produced free radicals. *Radiat. Res.,* **86,** 185.

WARD, J.F. (1985). Biochemistry of DNA lesion. *Radiat. Res.,* Suppl. **8,** S-103.

YARUS, M. & SINSHEIMER, R.L. (1964). Ultraviolet resistance of double-stranded φX174 DNA. *J. Mol. Biol.,* **8,** 614.