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Induction, repair and biological relevance of radiation-induced DNA lesions in eukaryotic cells*

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Summary. This report summarizes data on the induction, repair and biological relevance of five types of radiation-induced DNA lesions for which repair kinetic studies have been performed in eukaryotic cells by various laboratories. These lesions are: DNA-protein crosslinks, base damage, single-strand breaks, double-strand breaks and bulky lesions (clustered base damage in the nm-range). The influence of various factors, such as oxia/anoxia, linear energy transfer of the radiation used, incubation medium, cell cycle stage, thiol content, hyperthermia, on the induction and repair of these lesions is described. Radiation-sensitive cell lines are also included.

Introduction

A variety of lesions can be detected in the DNA of eukaryotic cells irradiated with ionizing radiation. These include DNA-protein crosslinks (DPCs), base alterations and base detachments, sugar alterations, bulky lesions, i.e. clusters of base damage, DNA single- and double-strand breaks. For most of these lesions, i.e. DNA-protein crosslinks, base damage, single- and double-strand breaks and bulky lesions, the kinetics of enzymatic repair has been determined. This paper is restricted to the induction of these lesions by ionizing radiation, their repair kinetics and biological relevance. It is an extended and updated version of an earlier publication (Frankenberg-Schwager 1989).

DNA-protein crosslinks

DNA-protein crosslinks are formed by covalent linkage between DNA and proteins of the nuclear matrix (e.g. Oleinick et al. 1986). Mainly those DNA regions which contain actively transcribing, and presumably also replicating, sequences are involved in the linkage to proteins, (e.g. Chiu et al. 1982; Oleinick

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et al. 1986). There is evidence that these DNA sequences are associated with the nuclear matrix (Ciejek et al. 1983; Robinson et al. 1983; McCready et al. 1982). A relatively high amount of about 6000 DPCs is observed per normal, unirradiated cell of Chinese hamster V79 lung fibroblasts in exponential growth (Oleinick et al. 1986). DPCs are induced linearly with radiation dose in the range of 10 to 100 Gy as shown for exponentially growing leukemia mouse cells (Cress and Bowden 1983) and Chinese hamster V79 cells (Chiu et al. 1984; Oleinick et al. 1986). γ-rays increase the number of new linkages between DNA and protein at a frequency of 150 DPCs per Gy per V79 cell (Ramakrishnan et al. 1987). Thus DPCs are induced at about a 4 time higher frequency than DNA double-strand breaks (Ramakrishnan et al. 1987). The same yield of radiation-induced DPCs is found in normal and radiation-sensitive cell lines (like *Ataxia telangiectasia* cells and the CHO mutant *xrs*-6 (Oleinick et al. 1990). At higher doses the number of DPCs approaches a plateau value which corresponds to the number of DNA attachment sites to the nuclear matrix (Ramakrishnan et al. 1987). The yield of DPCs in irradiated V79 cells is reduced in the presence of cysteamine (Radford 1986a). Based on the protective effect of the OH radical scavenger dimethyl-sulphoxide (DMSO), which is more pronounced in active DNA than in bulk DNA (Chiu et al. 1986), it appears that OH radicals are responsible for the production of DPCs in V79 cells (Oleinick et al. 1986). The yield of DPCs is enhanced when cells are irradiated under hypoxic conditions as shown for a human fibroblast cell line (Fornace and Little 1977), for CHO cells (Meyn and Jenkins 1984) and for mouse *L* and Chinese hamster V79 cells (Radford 1986a). Interestingly, DPCs are detected in cells from tissues irradiated in situ in acutely hypoxic (N_2-asphyxiated) mice and even in air-breathing animals (Meyn et al. 1986), indicating the presence of radiobiological hypoxic cells in tissues of air-breathing animals. Preirradiation hyperthermia (43° C for 15 min or longer) has no effect on the yield of radiation-induced DPCs in exponentially growing mouse leukemia cells (Cress and Bowden 1983) and in V79 cells (Radford 1986a), suggesting that the increased amount of nuclear protein after hyperthermia (Roti-Roti and Winward 1981) is not available for covalent bonding to the DNA damaged by ionizing radiation. In contrast, pre-irradiation hyperthermia increased the yield of DPCs in mouse *L* cells (Radford 1986a).

Mammalian cells are capable of repairing radiation-induced DPCs. DNA is removed from protein with biphasic kinetics during postirradiation incubation of cells at 37° C under growth conditions. Human fibroblasts in stationary phase from a confluent monolayer irradiated with 50 Gy under oxic or anoxic conditions exhibit a rapid component of repair of DPCs with a half-time constant $t_{1/2}$ of about 2 h and a slow component with a $t_{1/2}$-value of 12–13 h (Fornace and Little 1977). In actively transcribing DNA sequences, DPCs are not only preferentially induced but also the removal of DPCs in these regions is faster as compared to nontranscribing DNA (Oleinick et al. 1986). In agreement with these findings is the fast repair of DPCs in exponentially growing monolayer cultures of V79 cells irradiated with 60 Gy (Fig. 1). The $t_{1/2}$-value reported for the rapid component is 1 h which is followed by a slower component of repair (Chiu et al. 1984). Likewise, for growing mouse leukemia cells irradiated with 50 Gy, a rapid removal of DPCs with a $t_{1/2}$-value of 20 min is followed by a slower component with a $t_{1/2}$-value of 2 h (Cress and Bowden 1983). After preirradiation hyperthermic treatment of these cells, only the slow component

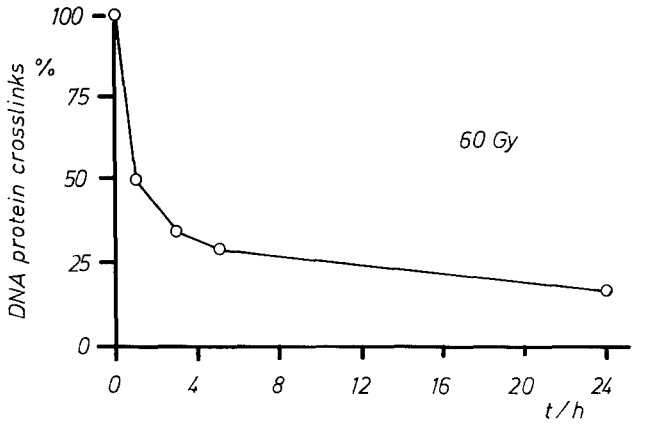

Fig. 1. Kinetics of DNA protein crosslink removal in Chinese hamster V79 lung fibroblasts after irradiation with a dose of 60 Gy (redrawn from Chiu et al. 1984)

of the repair kinetics can be detected (Cress and Bowden 1983). The slower removal of DPCs after preirradiation hyperthermia may be explained by the finding that the chemical nature of the bonding is changed with heat (Cress and Bowden 1983). Removal of DPCs is also observed in mouse C3H/10T1/2 fibroblasts which are held after irradiation with 50 Gy under nongrowth conditions at 37° C; in this case DPC removal is relatively slow with a $t_{1/2}$-value of 10–12 h (Fornace and Little 1977). DPCs are removed with the same efficiency in normal and radiation-sensitive (like AT and *xrs*-6) mammalian cell lines (Oleinick et al. 1990). Interestingly, Chinese hamster V79 fibroblasts remove γ-ray induced DPCs but not UV-induced DPCs (Chiu et al. 1984), which may point to a different structure of both types of DPCs. More residual DNA-protein crosslinks are observed in human melanoma cells incubated for 6 h after irradiation with high LET radiation (*N* ions, linear energy transfer (LET) = 530 keV/μm) compared to sparsely ionizing radiation (Eguchi et al. 1987). The mechanism of DPC removal appears to involve excision of the adduct, repair replication and a ligation step (Chiu et al. 1984). Poly-(ADP-ribose) plays a role in the release of DPCs, since 3-aminobenzamide, an inhibitor of the poly-(ADP-ribose) polymerase, slows down the second component of the DPC repair kinetics (Chiu et al. 1984). Cycloheximide has no effect on the repair of DPCs, suggesting that no newly synthesized proteins are required for DPC release (Chiu et al. 1984). Repair of DPCs seems to require an intact nuclear membrane, as indicated by its absence in metaphase cells (Oleinick et al. 1986).

The biological relevance of DPCs may depend on their chemical structure. To the normally occurring 6000 DPCs per V79 cell in exponential growth phase, only 200 DPCs are added by a mean lethal radiation dose (Ramakrishnan et al. 1987). Radiation-induced DPCs must be structurally different from normally occurring DPCs if they are involved in cell killing. However, evidence suggests that radiation-induced DPCs do not play a role in radiation-induced cell killing:

a) There is a lack of correlation between the increased yield of DPCs in hypoxic cells and the decreased radiation sensitivity of these cells relative to oxic cells (e.g. Fornace and Little 1977). Moreover, DPCs induced in oxic or anoxic cells are released with the same kinetics (Fornace and Little 1977).

b) Potentially lethal damage repair in irradiated C3H/10T1/2 mouse cells is completed within 6 h, whereas repair of DPCs under the same conditions continues at least up to 24 h (Fornace et al. 1980).
c) The same efficiency for DPC release is observed in normal and radiation-sensitive cells (Oleinick 1990).
d) Although preirradiation hyperthermia has a synergistic effect on cell killing, there seems to be no effect on the initial yield of DPCs. A possible relation between DPCs and cell killing could only refer to the decreased rate of DPC repair after hyperthermic treatment (Cress et al. 1983).
Radiation-induced DPCs may be relevant for cell aging, since there is a parallel in radiation- and age-induced changes of the properties of chromatin in mammalian cells (Cutler 1976).

Base damage

Damage to the four DNA bases is a major type of lesion caused by ionizing radiation in mammalian cells (Cerutti 1976). It is estimated that after a given dose the total number of damaged bases in human lung fibroblasts may be twice the number of DNA single-strand (SSBs) breaks (Cerutti 1974). Various radiation-induced alterations to the thymine base have been characterized (Cerutti 1974) which, in this paper, are all termed "thymine damage". A linear induction of thymine damage is observed for human lung fibroblasts irradiated with γ-rays up to 3000 Gy (Mattern et al. 1975), for EMT6 mouse sarcoma cells (up to 1000 Gy) (Dooley et al. 1984) and for HeLa cells irradiated with carbon ions up to about 10000 Gy (Mattern and Welch 1979). The efficiency of induction of thymine damage in HeLa and CHO cells is decreased with increasing linear energy transfer (LET) of carbon ions (LET range 170–780 keV/μm) (Mattern and Welch 1979). The frequency of induction of thymine damage depends on the chromatin structure. Thymine damage is about three times more frequently induced in replicating chromatin (Waters and Childers 1982), and a higher yield is also reported for actively transcribing chromatin of HeLa cells (Patil et al. 1985). Base damage induced by much lower doses can be monitored as endonuclease-sensitive sites (ESSs), i.e. base damaged sites incised by endonuclease yielding additional SSBs (Paterson 1978). However, only about half of the amount of radiation-induced base damage can be detected as ESSs, deduced on the one hand from the twice as frequent induction of total base damage relative to SSBs (Cerutti 1974) and on the other hand from the same frequency of ESSs and SSBs observed in human fibroblasts (Fornace et al. 1986). A linear relationship between induced ESSs and dose is found in mouse *L*1210 cells (Fornace 1982), in CHO cells (Van der Schans et al. 1979), in mouse *L* and Chinese hamster V79 cells (Radford 1986a) (Fig. 2). Thiol compounds have no effects on the yield of radiation-induced ESSs in CHO cells (Van der Schans et al. 1979) but cysteamine is reported to protect against ESSs in mouse *L* and V79 cells (Radford 1986a). The yield of base damage measured as ESSs is about equal under oxic or hypoxic conditions in irradiated human fibroblasts (Paterson et al. 1976), in CHO cells (Van der Schans et al. 1982a) and in mouse *L* cells (Radford 1986a). In contrast, the yield of ESSs in Chinese hamster cells is 2.5 times higher under oxic versus anoxic irradiation conditions (Skov et al. 1979). There is another paper reporting a high oxygen enhancement ratio (OER)

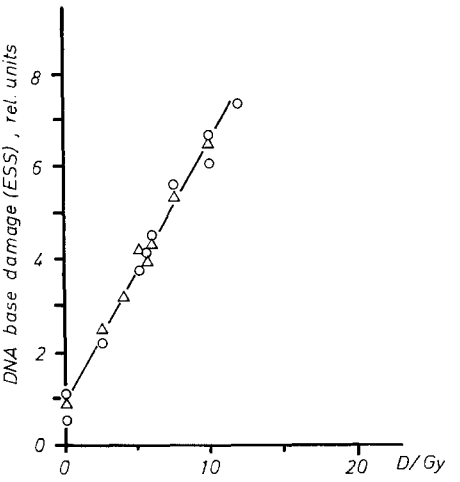

Fig. 2. Induction of base damage (measured as endonuclease-sensitive sites (ESS)) in irradiated mouse *L* (△) and Chinese hamster V79 (○) cells (redrawn from Radford 1986a)

of about 4 for the induction of ESSs in V79 cells, however, here also extremely high OER's for induced DNA single- (OER = 10) and double-strand breaks (OER = 6) have been measured (Kinsella et al. 1986). Fibroblasts from normal and *Xeroderma pigmentosum* individuals show the same efficiency of induction of ESSs (Fornace et al. 1986). Preirradiation hyperthermia has no effect on the induction of ESSs in mouse *L* cells (Radford 1986a). Recently, a powerful and sensitive method combining capillary gas chromatography with mass spectrometry with selected ion-monitoring was applied to detect base damage in irradiated cultured human lymphocytes (Dizdaroglu et al. 1987). At a dose range from 10 to 100 Gy the linear induction of a 8,5′-cyclo-2′-deoxyguanosine could be monitored. This type of damage arises by intramolecular cyclisation between guanine and the neighbouring sugar moiety and was not known before to occur in irradiated cells.

Thymine damage is removed from the DNA of cells during postirradiation incubation in growth medium at 37° C (Mattern et al. 1973, 1975; Mattern and Welch 1979; Paterson et al. 1976; Van der Schans et al. 1979). Repair of radiation-induced thymine damage is usually completed within 1 h and faster than repair of UV-induced thymine damage, which requires one to several hours for completion. For example, about 80% of thymine damage induced by a dose of 2500 Gy is removed from the DNA of human lung fibroblasts, SV40 transformed human fibroblasts and CHO cells within 15 min of postirradiation incubation (Mattern et al. 1975). Excision of thymine damage induced by ionizing radiation is equally efficient in human and rodent cells, in contrast to UV-induced thymine damage which seems to be reduced in the latter cells. This indicates that the endonucleolytic incision step differs for base damage induced by ionizing radiation and by UV-light. Endonucleolytic activity towards γ-irradiated DNA has been detected in human cells (Brent 1976) and in calf thymus cells (Bacchetti and Benne 1975). Radiation-induced base damage, monitored as ESS, is removed exponentially with time (first order reaction), with $t_{1/2}$-values of about 1 h in human fibroblasts (Paterson et al. 1976) and of about 1.5 h in *Xeroderma pigmentosum* fibroblasts (Fornace et al. 1986). The removal of ESS is impaired in a radiation-sensitive cell line of *Ataxia telangiectasia* patients

(Paterson et al. 1976; Paterson and Smith 1979) indicating a defective excision repair in these cells. Other AT cell lines, however, show normal excision repair (Hariharan et al. 1981; Van der Schans et al. 1981). The rate of ESS removal is not affected by the absence of oxygen and the presence of thiol compounds during irradiation of CHO cells (Van der Schans et al. 1979, 1982a). The chromatin structure of cells is important not only for the frequency of induction of thymine damage but also for the rate of repair. In active chromatin of HeLa cells, the rate of removal of thymine damage is higher than in inactive chromatin (Patil et al. 1985). Although high LET radiation reduces the yield of thymine damage, no effect on the rate of its removal is observed in HeLa cells (Mattern and Welch 1979). Base damage is removed from eukaryotic cells by two modes of excision repair. The "base excision repair" acts mainly on x-ray-induced base damage, whereas the "nucleotide excision repair" acts on damage induced by UV-light (e.g. Friedberg 1985).

There is evidence against the involvement of base damage in radiation-induced cell killing:

a) Lack of correlation between cell killing and base damage induction in dependence of LET. For example, for carbon ions the efficiency for cell killing is maximum at a LET-value of about 200 keV/μm, yet the yield of thymine damage is reduced by a factor of 5 (Mattern and Welch 1979). Furthermore, the rate and extent of repair is the same after high and low LET radiation (Mattern and Welch 1979).

b) Hypoxia protects against radiation-induced cell killing but, in most cases, not against base damage (Paterson et al. 1976; Radford 1986a; Van der Schans et al. 1982a).

c) Some radiation-sensitive AT cell lines are capable of repairing ESSs (Hariharan et al. 1981; Van der Schans et al. 1981), others are not (Paterson et al. 1976; Paterson and Smith 1979).

d) Hyperthermia enhances radiation-induced cell killing but has apparently no effect on the yield of base damage, measured as ESSs (Radford 1986a).

Radiation-induced base damage plays a role in the induction of point mutations. For example, 84% of the mutational events in the essential gene (*aprt*) of Chinese hamster cells are classified as "point mutations" (Grosovsky et al. 1987). In contrast, mainly large deletions rather than point mutations given rise to mutational events in a non-essential gene (*hprt*) of V 79 cells (Thacker 1986). Thymine damage may play a role in the process of cellular ageing. With increasing age of human WI-38 cells in culture, the efficiency of removal of radiation-induced thymine damage decreases and hence an accumulation of thymine damage in the DNA of old cells is observed (Mattern and Cerutti 1979).

DNA single-strand breaks

Induction of SSBs has been studied for a large variety of mammalian cells and has been found to increase in proportion to the dose of radiation (e.g. Ahnström and Edvardsson 1974; Coquerelle et al. 1973; Kohn et al. 1976; Roots et al. 1979). The same induction frequency for SSB is observed in radiation-resistant Chinese hamster and in radiation-sensitive mouse cells (Sakai and Okada 1984; Radford 1986a). Likewise, no difference in SSB induction is detected in normal human fibroblasts and in radiation-sensitive cells from retinoblastoma

patients (Woods et al. 1982). The efficiency of SSB induction decreases with increasing LET as shown for Chinese hamster V79 cells irradiated with high (100–600 keV/μm) and low (0.5–2.4 keV/μm) LET radiation (Ritter et al. 1977). For human skin fibroblasts the efficiency of SSB induction by 14.6 MeV neutrons is 30% less than by γ-rays (Van der Schans et al. 1983) and also for mouse cells a decreased induction frequency of SSB is reported after irradiation with fast neutrons (6 MeV) (Sakai et al. 1987). Hypoxia decreases the yield of radiation-induced SSBs relative to oxic conditions (Palcic and Skarsgard 1972; Lennartz et al. 1975). The decrease is by a factor of 3 for Chinese hamster cell V79 fibroblasts (Koch and Painter 1975) and for human fibroblasts (Revesz 1985), but also a factor of about 4 has been reported for Chinese hamster cells (Hohman et al. 1976). Radiation-induced SSBs, assayed under alkaline conditions, comprise about 30% or 50% alkaline labile sites in rat thymocytes irradiated under oxic or anoxic conditions, respectively (Lennartz et al. 1973). Exogenously supplied thiol compounds reduce the yield of SSBs in human fibroblasts (Van der Schans et al. 1979) and in mouse and Chinese hamster cells (Radford 1986a) irradiated under hypoxic conditions. In agreement with these findings is the increased yield of SSBs in irradiated anoxic human fibroblasts depleted in endogenous glutathione (Edgren et al. 1981). Recently, it was reported that sensitization to SSB induction occurs in HeLa and CHO cells depleted of intracellular thiol compounds at moderate oxygen concentrations (0.5–10 μM), whereas sensitization under oxic or extreme hypoxic conditions (0.01 μM oxygen) is marginal (Van der Schans et al. 1986). The efficiency of SSB induction does not vary during the cell cycle (Graubmann and Dikomey 1983). Preirradiation hyperthermia has no effect on the yield of SSB in CHO cells (Dewey et al. 1980), HeLa cells (Lunec et al. 1981) and mouse cells (Radford 1986a). Contradictory to this, an increased frequency of radiation-induced SSBs following hyperthermic treatment of CHO cells is observed (Corry et al. 1977; Mills and Meyn 1981). SSBs are preferably induced by radiation in transcriptionally active DNA sequences as shown in mouse (Chiu et al. 1982) and Chinese hamster (Oleinick et al. 1985) cells. This is explained by the better accessibility of OH radicals to DNA during transcription.

Repair of radiation-induced SSBs has been studied in many different cell lines. SSBs are rejoined exponentially with time (first order reaction) during postirradiation incubation of cells under growth conditions at 37° C (Fig. 3) (e.g. Dikomey and Franzke 1986). Up to three components of the SSB repair kinetics can be distinguished. The first component comprises 70–90% of all SSBs which are rejoined independently of dose with a $t_{1/2}$-value between 2 and 10 min. The induction of these rapidly reparable SSBs can be prevented by the presence of cysteamine during irradiation (Van der Schans et al. 1982a). The second, slower component represents a dose-dependent rejoining of SSBs with $t_{1/2}$-values of about 10 min and more (e.g. Bryant et al. 1984; Dikomey and Franzke 1986; Wheeler and Wierowski 1983; Wheeler and Nelson 1987). This component may represent a mixture of SSB rejoining and dose-dependent formation of new SSBs due to incisions at base damaged sites (Bryant et al. 1984; Dikomey and Franzke 1986). The third component is attributed to the rejoining of DNA double-strand breaks with a $t_{1/2}$-value $\geq$ 1 h (Bryant and Blöcher 1980). SSBs are rejoined with the same rate in radiation-resistant and -sensitive human cell lines (Paterson et al. 1976; Hariharan et al. 1981; Woods et al. 1982; Van der Schans et al. 1982a, 1983) and rodent cells (Sakai and

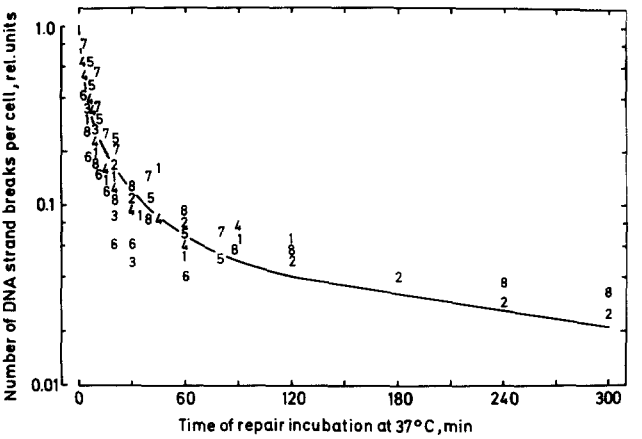

Fig. 3. Rejoining kinetics of radiation-induced total DNA strand breaks measured in various studies. The figure is taken from Dikomey and Franzke 1986. Data was taken from 1: V79, 9 Gy (Ahnström and Edvardsson 1974); 2: Ehrlich ascites, 100 Gy (Bryant and Blöcher 1980); 3: mouse, 3 Gy (Bowden and Kasunic 1981); 4: Ehrlich ascites, 6 Gy (Jorritsma and Konings 1983); 5: V79, 100 Gy (Körner et al. 1978); 6: NHIK, 18.5 Gy (McGhie et al. 1983); 7: human lymphocytes, 1 Gy (McWilliams et al. 1983); 8: V79, 45 Gy (Sakai and Okada 1981); Solid line: CHO, 3 and 9 Gy (Dikomey and Franzke 1986)

Okada 1984). However, in the latter case the fraction of unrejoined SSBs is higher in radiation-sensitive cells. No difference in the rate of SSB rejoining is observed in undifferentiated and terminally differentiated rodent brain cells, yet the slower overall SSB rejoining rate in differentiated neurons is mainly due to the increased fraction of slow reparable breaks in these cells (Wheeler and Nelson 1987). Contact inhibited mouse C3H/10T1/2 cells in stationary phase exhibit a slow rejoining of SSBs when cells are kept after irradiation under nongrowth conditions. 90% of the induced SSBs are rejoined during the first hour and most of the remaining SSBs disappear during the next 5 h (Fornace et al. 1980). The rate of SSB rejoining after irradiation of CHO cells with high LET α-particles is decreased compared to low LET irradiation (Cole et al. 1980). In contrast, a similar rate of SSB rejoining is observed for both human fibroplasts and cells from AT patients after high LET (14.6 MeV neutrons) and low LET (^{60}Co γ) irradiation (Van der Schans et al. 1983) and also for human lymphocytes (McWilliams et al. 1983). In Chinese hamster cells the fraction of nonrejoined SSBs increases with the LET of heavy ions from 1.4% (at 1 keV/μm) to 18% (at 1953 keV/μm) (Ritter et al. 1977; see also Roots et al. 1979). An increased yield of unrejoined SSBs after high LET irradiation is also reported for Chinese Hamster V79 cells irradiated with fast neutrons (Ahnström and Edvardsson 1974; Körner et al. 1978), for CHO cells, where 20% of the SSBs induced by α-particles remained unrejoined after 6 h compared to only 10% after low LET irradiation (Cole et al. 1980), and in mouse cells irradiated with fast neutrons (Sakai et al. 1987). There is no correlation of the rate of SSB rejoining with the cycle dependent variation in radiosensitivity (Sawada and Okada 1970), but a similar cell cycle dependent variation of nonrejoined SSBs and cellular radiosensitivity is observed (Sakai and Okada 1984). On the other hand, the rate of SSB rejoining was found to be dependent on the chroma-

tin structure of irradiated cells. For example, a slower rate of rejoining is measured in Chinese hamster fibroblasts in metaphase compared to asynchronous cells (Oleinick et al. 1985). Radiation-induced SSBs are rejoined even under extreme hypoxic conditions in Chinese hamster V79 cells, but the amount of SSBs left unrejoined after an incubation period of one hour is 20% in cells under extreme hypoxia and only 10% under oxic conditions (Koch and Painter 1975). Treatment of cells at temperatures of about 43° C prior to irradiation reduces the rate and the extent of SSB rejoining as shown for CHO cells (Clark and Lett 1976; Mills and Meyn 1981), HeLa cells (Lunec et al. 1981), mouse cells (Bowden and Casunic 1981) and Ehrlich ascites tumour cells (Jorritsma and Konings 1983). The rate and extent of SSB rejoining may depend on the level of intracellular thiol compounds. Rejoining of SSBs within one hour after irradiation of human fibroblasts under oxic conditions is only 70% in glutathione-depleted cells compared to glutathione-proficient cells, whereas rejoining of SSBs radiation-induced under hypoxia is about 100% in both cell lines (Edgren et al. 1981; Revesz and Edgren 1984). In contradiction to this, no effect of thiol depletion on postirradiation rejoining of SSBs has been reported (Vos et al. 1986).

There is evidence that SSBs are not involved in cell killing:

a) With increasing LET of radiation the efficiency for cell killing increases (e.g. Fowler 1981), yet the efficiency for SSB induction decreases (Ritter et al. 1977; Roots et al. 1979; Van der Schans et al. 1983; Sakai et al. 1987).

b) The rate of rejoining of the majority of SSBs is about equal after high and low LET radiation (Van der Schans et al. 1983; Sakai et al. 1987) and not related to cellular radiosensitivity (e.g. Szumiel 1981).

c) A level of SSBs, induced by hydrogen peroxide via OH radicals, corresponding to 10 Gy does not result in killing of Chinese hamster cells and does not affect cell inactivation by γ-rays (Ward et al. 1985).

However, a correlation between unrejoined SSBs and cell killing is observed for radiation-resistant and -sensitive cell lines (Sakai and Okada 1984), for the cell cycle dependency (Sakai and Okada 1984), for the LET dependency (Cole et al. 1980; Ritter et al. 1977), and for preirradiation hyperthermia (Clark and Lett 1976; Bowden and Casunic 1981; Lunec et al. 1981; Mills and Meyn 1981). It remains to be elucidated to which extent DNA double-strand breaks contribute to the amount of unrejoined SSBs (see Ritter et al. 1977; Dikomey and Franzke 1986; Radford 1986a; Sakai et al. 1987).

DNA double-strand breaks

After irradiation with supralethal doses, a linear relationship between double-strand breaks (DSBs) and dose has been observed for various types of eukaryotic cells (e.g. Corry and Cole 1968; Lehman and Ormerod 1970; Frankenberg-Schwager et al. 1979; Blöcher 1982; Van der Schans et al. 1982a). Contradictory to this, in cases where neutral filter elution was used to measure DSBs, it was reported that a non-linear, shoulder-type induction curve for DSBs in rodent cells is found at low doses as applied in survival studies (Radford 1985; Prise et al. 1987). Controversial opinions exist about the possibility that this finding is a technical artefact depending on the cellular lysis conditions applied (Okayasu and Iliakis 1989; Radford 1990). However, in yeast cells, DSBs are induced

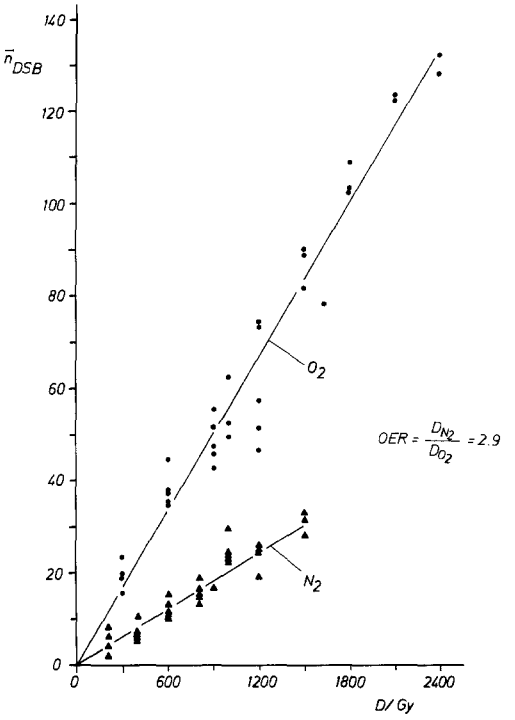

Fig. 4. Induction of double-strand breaks in yeast cells irradiated under oxic or anoxic conditions. $\bar{n}_{DSB}$: number of DSB per cell (redrawn from Frankenberg-Schwager et al. 1979)

under various irradiation conditions linearly with dose in a dose range applied in survival assays (Fig. 4) (Frankenberg-Schwager et al. 1979; Frankenberg et al. 1986). Yeast mutants, exhibiting no detectable rejoining of DSBs, show an exponential survival curve where about one DSB per cell corresponds to one lethal event (Frankenberg et al. 1981). On the basis that one unrepaired DSB is lethal for a yeast cell, a curvi-linear induction of DSB with dose should result in a shoulder-type rather than an exponential survival curve of such DSB repair deficient mutants. Moreover, measuring chromosome breaks by the premature chromosome condensation (PCC) technique, a linear relationship between breaks and dose is observed in the dose range up to 20 Gy (Waldren and Johnson 1974) but also at lower doses (Cornforth and Bedford 1983; Pantelias and Maillie 1985). The yield of radiation-induced DSBs is lower by a factor of about 25 than the yield of SSBs based on 1000 SSBs per mammalian cell per Gy (Elkind 1979) and 40 DSBs per cell per Gy (Blöcher 1982). In oxic cells the yield of radiation-induced DSBs is about three-fold higher than in anoxic cells (Fig. 4) (e.g. Lennartz et al. 1975; Kampf et al. 1977a; Frankenberg-Schwager et al. 1979; Radford 1985; Prise et al. 1987). The yield of DSBs (measured by the neutral sedimentation technique) increases with increasing LET of the radiation applied (Kampf et al. 1977; Cole et al. 1980; Frankenberg et al. 1981; Van der Schans et al. 1983). In agreement with this, Ne ions (LET = 183 keV/μm) are found to be 1.5 times more effective at producing chromatin breakage (assayed by the PCC technique) compared to x-rays (Goodwin et al. 1989). In contrast, virtually the same induction frequency for DSBs is observed for sparsely ionizing radiations and α-particles, when the neutral filter elution rather than the neutral sedimentation technique was applied for DSB measurement (Coquerelle et al.

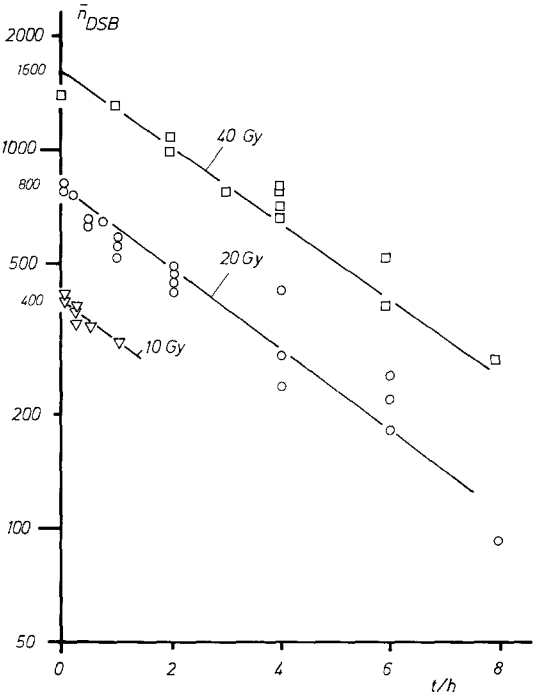

Fig. 5. Kinetics of double-strand break rejoining under nongrowth conditions in Ehrlich ascites tumor cells irradiated in stationary phase with 10, 20 and 40 Gy, $\bar{n}_{DSB}$: number of DSB per cell (redrawn from Blöcher and Pohlit 1982)

1987; Prise et al. 1987). Preirradiation hyperthermic treatment of rodent cells is reported to yield an increased induction frequency of DSBs (Radford 1985, 1986b). No difference between radiation-resistant and -sensitive cell lines is found for the induction of DSBs (Van der Schans et al. 1982a, 1983) and for chromosome breakage (Cornforth and Bedford 1985). However, in one study a higher yield of initial DSBs is observed in radiation-sensitive cell lines compared to -resistant cells (Radford 1986b). The effect of glutathione-depletion on the yield of DSBs is relatively small in CHO cells irradiated under oxic or severely hypoxic conditions; only at moderate hypoxia does glutathione-depletion sensitize for DSB induction (Van der Schans et al. 1986). In rodent cells, a protective effect of cysteamine against radiation-induced DSBs is observed (Radford 1986b).

DSBs are rejoined during postirradiation incubation in various types of cells under growth or nongrowth conditions with monophasic (Bradley and Kohn 1979; Bryant and Blöcher 1980) or biphasic (Cole et al. 1980; Van der Schans et al. 1982a, 1983; Coquerelle et al. 1987) kinetics. The rejoining of radiation-induced DSBs is exponential with time (first order reaction) and independent of dose as shown for mouse cells (Bradley and Kohn 1979) and Ehrlich ascites tumour cells (Fig. 5) (Blöcher and Pohlit 1982). Great variations in the half-time constants $t_{1/2}$ are observed for DSB rejoining under growth conditions, which may depend on the method of DSB measurement: an initial rapid component, with a $t_{1/2}$-value of 3–10 min (Cole et al. 1980; Van der Schans et al. 1983; Weibezahn and Coquerelle 1981) is followed by a slow component, the $t_{1/2}$-value of which ranges from 40 min to 4 h (Lehman and Stevens 1977; Bradley and Kohn 1979; Bryant and Blöcher 1980). DSB rejoining in stationary Ehrlich ascites tumour cells is slower under nongrowth conditions ($t_{1/2} = 3$ h) compared to growth conditions ($t_{1/2} = 1.8$ h) (Bryant and Blöcher 1980). A close correlation

exists between DSB rejoining and rejoining of chromosome breaks monitored by the PCC technique. The biphasic, dose-independent rejoining of chromosome breaks under nongrowth conditions shows an exponential dependence of time with $t_{1/2}$-values of 2 h and 6 h for the fast and slow component, respectively (Cornforth and Bedford 1983). The rate of DSB rejoining is independent of the cell cycle phase in which cells are irradiated (Weibezahn and Coquerelle 1981; Blöcher et al. 1983). The same rate of DSB rejoining is observed for radiation-resistant and most -sensitive cell lines (Lehman and Stevens 1977; Coquerelle and Weibezahn 1981; Van der Schans et al. 1982b, 1983) but few radiation-sensitive lines do exhibit an impaired rate of DSB rejoining (Coquerelle and Weibezahn 1981). Radiation-sensitive cells from *Ataxia telangiectasia* patients with normal rate of DSB rejoining show, however, a higher fraction of nonrejoined chromosome breaks (Taylor 1978; Cornforth and Bedford 1985). After high LET irradiation the rate of DSB rejoining may be (Bryant and Blöcher 1980; Coquerelle et al. 1987; Blöcher 1988; Frankenberg-Schwager et al. 1990) or may not be slower (Van der Schans et al. 1983) compared to low LET irradiation. In the course of DSB rejoining after low and high LET irradiation the linear relationship between initial DSB and dose is converted into a linear-quadratic relationship between unrejoined DSB and dose (Frankenberg-Schwager et al. 1980a, b, 1982; Blöcher and Pohlit 1982; Blöcher 1988; Frankenberg-Schwager et al. 1990) and the fraction of unrejoined DSBs is higher after high LET irradiation (Cole et al. 1980; Coquerelle et al. 1987; Frankenberg-Schwager et al. 1982; Blöcher 1988; Frankenberg-Schwager et al. 1990). Similar findings were reported for interphase chromatin breaks (PCC fragments) after irradiation of noncycling, diploid human fibroblasts with low doses of x-rays: Initial PCC fragments depend linearly on dose, whilst residual PCC fragments (present after a 24 h incubation of cells under nongrowth conditions) show a non-linear dependence on dose (Cornforth and Bedford 1987). Likewise, the fraction of unrejoined chromatin fragments was higher in a human/hamster hybrid cell-line irradiated with Ne ions compared to x-rays (Goodwin et al. 1989). Hyperthermic treatment reduces the rate and extent of rejoining of radiation-induced DSBs (Corry et al. 1977; Ben-Hur et al. 1978; Dikomey 1982). No effect of thiol depletion on the rejoining of DSBs is reported for CHO cells irradiated under oxic or hypoxic conditions (Vos et al. 1986). No difference in the time course of DSB rejoining was observed after a 2 h incubation in growth medium of primary human skin fibroblasts irradiated in the presence of air, N_2 or N_2 + cysteamine (20 m*M*) (Van der Schans et al. 1982). The mechanism of rejoining of radiation-induced DSBs seems to involve a recombinational process (Resnick 1976) but other authors suggest that at least part of the DSBs are rejoined by simple ligation (Weibezahn and Coquerelle 1981). In fact, there is experimental evidence for the occurrence of the heteroduplex postulated to be formed during the recombinational repair of DSBs in mammalian cells (Resnick and Moore 1979; Fonck et al. 1984).

Evidence exists that radiation-induced DSBs lead to cell killing:

a) With increasing LET of the radiation both cell killing and the yield of unrejoined DSBs increase (Cole et al. 1980; Frankenberg-Schwager et al. 1982; Coquerelle et al. 1987; Blöcher 1988; Frankenberg-Schwager et al. 1990).

b) Radiation-sensitive human fibroblasts from *Ataxia telangiectasia* patients show a higher level of unrejoined chromosome breaks compared to normal cells (Taylor 1978; Cornforth and Bedford 1985).

c) Yeast mutants, completely deficient in DSB rejoining, are killed by about one DSB per cell (e.g. Frankenberg et al. 1984a). They do not repair potentially lethal damage (Petin 1979; Reddy et al. 1982; Frankenberg-Schwager et al. 1985, 1987) and sublethal damage (Reddy et al. 1982; Frankenberg et al. 1984b; Frankenberg-Schwager et al. 1985). Furthermore, these mutants do not show a dose-rate effect (Reddy et al. 1982), which is in agreement with the finding that the better survival of normal cells at low dose-rate irradiation is correlated with the rejoining of DSBs during irradiation (Frankenberg-Schwager et al. 1981). DSBs (Kampf et al. 1977; Cole et al. 1980; Frankenberg et al. 1981; Van der Schans et al. 1983; Frankenberg et al. 1986) seem to be involved in chromosome abberration production (e.g. Bauchinger et al. 1975; Edwards et al. 1980; Zoetelief and Barendsen 1983; Thacker et al. 1986) and cell transformation (Borek et al. 1978; Han and Elkind 1979; Robertson et al. 1983; Barendsen and Gaiser 1985; Hall and Hei 1985), based on a similar LET-dependence for all three endpoints.

There is no evidence for the involvement of DSB in the formation of point mutations based on the fact that DSB repair-proficient and -deficient yeast cells exhibit the same mutation frequency (Magni et al. 1977).

Bulky lesions

Bulky lesions are defined as clusters of base damage involving at least three unpaired bases, not necessarily combined with a strand break (Silber and Loeb 1982; Kohfeldt et al. 1988). These lesions can be detected by the DNA single-strand specific endonuclease S1 resulting in the formation of DSBs. The induction of bulky lesions (S1-sites) by ionizing radiation depends on the structure of DNA: These lesions do not occur in DNA irradiated in vitro, but they are found in the DNA of irradiated yeast (Andrews et al. 1984; Geigl 1987) and mammalian cells (Yoshizawa et al. 1978; Furuno et al. 1979). Bulky lesions are induced in proportion to the radiation dose at the same or somewhat higher frequency as DSBs (Yoshizawa et al. 1978; Andrews et al. 1984; Geigl 1987). The yield of bulky lesions is reduced by approximately 50% in mouse leukemia

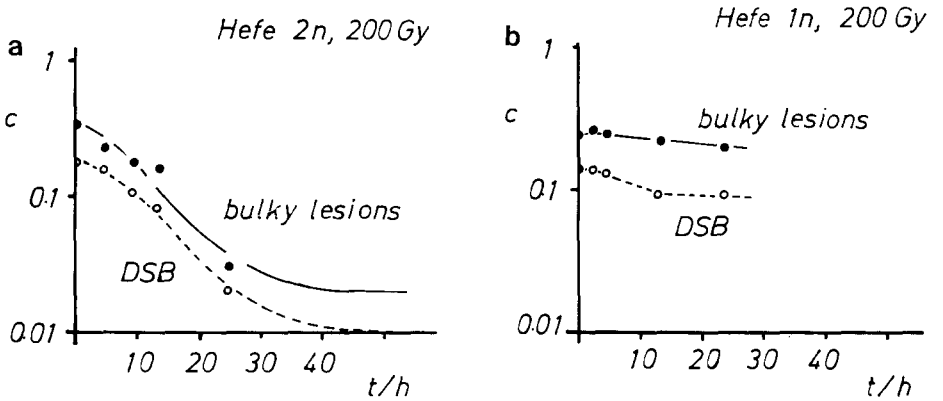

Fig. 6. Repair under nongrowth conditions of bulky lesions and double-strand breaks (DSB) in diploid **(a)** and haploid **(b)** yeast cells irradiated with 200 Gy. C: Radiation-induced lesions per DNA molecule of band 13/12, S1: endonuclease S1, (redrawn from Geigl 1987)

cells irradiated with cyclotron neutrons (30 MeV) compared to γ-rays (Furuno et al. 1979).

Bulky lesions disappear from the DNA of yeast cells at about the same rate as DSBs (Fig. 6a) (Geigl 1987). The mechanism involved in the repair of bulky lesions in yeast seems to require a recombinational process, since haploid cells in stationary phase are not capable of removing neither bulky lesions nor DSBs (Fig. 6b) (Geigl 1987). The biological significance of radiation-induced bulky lesions remains to be elucidated.

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