Radioadapted Chicken Embryo Cells: Challenge Specificity and Alterations in Higher-Order DNA Structure

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Radioadapted chicken embryo cells (X-irradiation *in ovo* with 10 cGy at the 14th day of development with priming periods of 24 h) were treated *in vitro* by challenge doses of 14 different DNA- and/or chromatin-interactive agents, including X-rays. A decrease in the cellular damage, as measured by scheduled DNA synthesis, was only observed with X-irradiation. Sedimentation of nucleoids as well as viscosity of alkaline lysates from ethidium bromide (0.35–400 μg/ml)-, novobiocin (125–1800 μg/ml)-, and hyperthermia (30 min at 43° and 45°)-treated cells suggest a higher tendency of radioadapted cells to undergo positive DNA supercoiling. When DNA from adapted and non-adapted chicken embryo cells was used as substrate, neither its digestion by DNase I nor the inhibition of DNase I activity by various DNA-interactive agents was changed in primed cells. From the previous investigations as well as from the present results it is concluded that an increase of tightening of protein-DNA interactions within the nuclear matrix is a molecular determinant of the elevated radiation resistance in radioadapted chicken embryo cells.

Introduction

From investigations comprising prokaryotic and eukaryotic cells as well as non-mammalian and mammalian systems evidence increases that, under specialized conditions, small doses of ionizing radiation are able to induce some radiation resistance (for review see *e.g.,* Wolff, 1992; Joiner, 1994). When human lymphocytes, for instance, were exposed to small doses (within the order of 1–10 cGy) of X-rays or to [^{3}H]thymidine, the cells showed reduced radiation sensitivity with respect to chromtid breaks induced by a subsequent acute "challenge"-irradiation with 1–5 Gy (for review see Shadley, 1994). In general, the increase in radiation resistance following a pre-exposure to low doses of ionizing radiation (= adaptive, priming and/or conditioning exposure) is called an adaptive response. Since various agents, *e.g.,* hydrogen peroxide (Wojewodzka *et al.,* 1994) and alkylating agents (Anderson *et al.,* 1988) exhibited priming activity, and since the susceptibility of cells to, *e.g.,* alkylating agents (Osmak, 1988), mitomycin C (Osmak and Horvat, 1992) and bleomycin (Zhou *et al.,* 1993) could be reduced by low doses of gamma-rays, neither the conditioning agent nor the primed response seem to be limited to ionizing radiation.

A radioadaptive response of the chicken embryo has been observed in previous investigations (Tempel and Schleifer, 1995). When chicken embryos were X-irradiated *in ovo* with 5–30 cGy (= priming dose) at the 13th–15th day of development, significantly less radiation damage was observed when the response to a challenge exposure (within the order of 4–32 Gy) was measured by scheduled DNA synthesis, nucleoid sedimentation and viscosity of alkaline cell lysates 13–36 h following the priming exposure. Since an increase in DNA repair capacity could be excluded as the sole and dominant mechanism of the adaptive response, a reduction of the initial radiation damage in primed cells has been postulated. Preliminary tests revealed changes in the sedimentation of nucleoids of radioadapted cells (Tempel and Schleifer, 1995). Considerable evidence exists that nuclear and chromatin structures are molecular determinants of the cellular radiosensitivity (see, *e.g.,* Olive, 1992; Schwartz and Vaughan, 1993; McMillan and Peacock, 1994; Oleinick and Chiu, 1994). Presently, extensive studies were performed therefore with respect to changes in higher-order

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DNA and/or chromatin structures in radioadapted chicken embryo cells. Additional experiments were carried out with respect to the challenge specificity of the radioadaptive response comprising various DNA- and/or chromatin-interactive chemical and physical agents. The methods available comprised scheduled DNA synthesis, nucleoid sedimentation, viscometry of alkaline cell lysates and enzymatic analyses in highly purified DNA of unprimed and primed chicken embryo brain cells.

Materials and Methods

Chemicals

Bleomycin, doxorubicin, and cisplatin were gifts from Mack (Illertissen, Germany), Farmitalia Carlo Erba (Freiburg, Germany), and Rhône-Poulenc (Köln, Germany), respectively. [Methyl-^{3}H]thymidine (dT-^{3}H, specific activity 3.2 TBq/mmol) was obtained from DuPont NEN, DuPont de Nemours, Bad Homburg, Germany). All other chemicals were from Merck (Darmstadt, Germany), Serva (Heidelberg, Germany), Sigma (München, Germany), and Aldrich (Steinheim, Germany).

Chicken embryos and cell suspensions

Fertilized White Leghorn eggs obtained from a local hatchery were placed in an incubator for 15 days at 37 °C and 60% relative humidity. At the 15th day of development, the embryos were removed from the eggs and killed by decapitation. Brain and/or liver cell suspensions were prepared as described in detail previously (Ignatius and Tempel, 1992) and adjusted to final concentrations of about 10^6–10^8 cells/ml medium (Hank's solution, Ca^{2+}, Mg^{2+}-free).

Exposure of the embryos and cell suspensions

At the 14th day of development, the embryos were distributed randomly in two groups: one group served as the unprimed controls (sham-irradiation), while the other one was pre-irradiated *in ovo* with low doses of X-rays (in general with 10 cGy = priming = inducing = adaptive exposure). X-irradiation was performed as described previously (Tempel and Schleifer, 1995). 24 hours later, the cell suspensions were exposed to physical or chemical agents, depending on the type of the experiment. The physical agents comprised UV- and X-irradiation or hyperthermia (30 min at 43 or 45 °C). For testing chemical agents, stock solutions of the test substances were freshly prepared in Hank's solution at 11-times the concentration needed in the cell suspensions. In general, 0.1 ml of the stock solutions were added to 1 ml of the cell suspensions. The addition of the test substances was followed by a 30-min incubation of the cells at 37 °C prior to analytical procedures.

Analytical procedures

The *nucleoid sedimentation* technique and *viscometry of alkaline cell lysates* were performed as outlined in previous papers (*e.g.,* Tempel and Schleifer, 1995). *DNA synthesis* was measured by the uptake of dT-^{3}H into the perchloric acid (6%) precipitate of the cells (Tempel and Schleifer, 1995). *DNase I activity* was estimated by the increase in absorbance of the whole reaction mixture at 260 nm during an incubation period of 20 min (Uvikon spectrometer 930, Kontron Instruments, Eching, Germany). The enzyme assay consisted of 800 µl Tris(hydroxymethyl)-aminomethan–HCl, pH 7.4, 100 µl DNase I solution (1.357 Kunitz units) in aq. bidest., 50 µl of an aqueous solution of DNA (100 µg/assay, isolated previously from chicken embryo brains) and 50 µl 4 M $MgCl_2$. By incubating the enzyme assay at 22 °C, an almost linear increase in the absorbance of the reaction mixture measured automatically at 260 nm was produced for 2.5–5.0 min.

The *O^6-alkylguanine-DNA alkyltransferase (AT)-activity* was estimated as described previously (Link and Tempel, 1991) by quantifying [^{3}H]-methyl transferred from the substrate, *i.e.,* DNA-O^6-[^{3}H] methylguanine, to the AT acceptor protein. For the *DNA isolation* the rapid method of Lahiri and Nurnberger (Lahiri *et al.,* 1992), that allows the extraction of high quality deoxyribonucleic acid without the use of phenol/chloroform and without enzymatic digestion, was used. When diluted to a concentration of about 100–150 µg/ml, the DNA extracted from chicken embryo brain cells exhibited absorption ratios 260/280 nm of about 1.7. Using the Bradford method (BioRad Kit, München, Germany), no protein contamination could be detected in the extracts. Both param-

eters indicate that the extracts are rather free from protein and/or RNA contamination (Lahiri *et al.*, 1992).

Moreover, the hydrolysates of the extracts exhibited, when analysed by high-performance-liquidchromatography, no extra peaks or other significant differences in their chromatograms as compared to calf thymus DNA (Aldrich, Steinheim, Germany).

In general, each data point represents the mean of at least two to three independent experiments which were carried out in triplicate.

Results

To obtain some information about the challenge specificity of the adaptive response of chicken embryo cells to X-irradiation, the influence of various DNA- and/or chromatin-interactive agents on controls and radioadapted brain cells was tested *in vitro* by determining scheduled DNA synthesis, nucleoid sedimentation, and viscometry of alkaline cell lysates. The results presented in Table I show that UV-light as well as a number of substances exhibiting different mode of action on DNA- and/or chromatin revealed no significant differences between primed and non-primed cells. A significant elevation of nucleoid sedimentation, however, was seen in radioadapted cells when subjected to ethidium bromide (EB, 50–400 μg/ml), novobiocin (NB, 180 μg/ml), and hyperthermia (30 min at 43 and 45 °C) immediately before the sedimentation assay. In general, the increase in nucleoid sedimentation from radioadapted brain cells was accompanied by a depletion of the viscosity of alkaline cell lysates (Table II).

In a further series of experiments, radioadapted and control cells were incubated with EB (30 min at 37 °C) at concentrations of 0.35–400 μg/ml. Subsequently, the nucleoid sedimentation was measured. Preliminary results (Tempel and Schleifer, 1995) could be confirmed: In radioadapted brain- and liver-cells (pre-exposure *in ovo* with 10 cGy), nucleoid sedimentation was enhanced by 20–40% at EB-concentrations of 50–

Table I. Scheduled DNA synthesis (SDS), nucleoid sedimentation (SED), and viscosity of alkaline lysates (VISC) of chicken embryo brain cells immediately following *in vitro*-exposure to DNA- and/or chromatin-interactive agents. With respect to previous investigations in chicken embryo cells (Tempel *et al.*, 1992), the agents were tested at 3–4 different doses resulting in a SDS-inhibition by about 10–20% to 80–90% (if available). For practical reasons (extreme sensitivity of the SED- and VISC-test to bleomycin) or reasons given in the text, bleomycin, ethidium bromide and novobiocin comprised dose-ranges of 0.6–2.4 μg/ml, 50–400 μg/ml, and 125–250 μg/ml, respectively, in the SED- and VISC-assays. For each dose/concentration, the values estimated in radioadapted cells were divided by the values estimated in non-adapted cells. The values listed in the table represent the mean of the ratios (Q) obtained (± standard deviation). Sham-irradiated controls: SDS: $(1.1 \pm 0.2) \times 10^6$ dpm/μg DNA, SED: 1.8 ± 0.17 cm/2 h, VISC: 44 ± 9 s (= elution time of 3 ml through a 6 cm capillary with a diameter of 0.8 cm).

Agent	Concentrations used [μg/ml]	Q Non-adapted/adapted cells SDS	SED	VISC
–	–	1.00 ± 0.12	1.00 ± 0.05	1.00 ± 0.15
Bleomycin	50–500	1.13 ± 0.11	1.16 ± 0.08	1.01 ± 0.15
Cadmium·Cl_2	10^3–10^4	0.89 ± 0.12	1.08 ± 0.10	1.02 ± 0.16
Cisplatin	12.5–100	0.92 ± 0.21	1.12 ± 0.09	0[a]
Cystein·HCl	100–400	0.97 ± 0.10	1.04 ± 0.12	1,04 ± 0.15
Doxorubicin	1.25–10	0.99 ± 0.14	0.97 ± 0.14	0.98 ± 0.18
Ethidiumbromide	10–300	0.94 ± 0.11	1.30 ± 0.10[b]	0.71 ± 0.19[b]
Hoechst 33258	12.5–100	0.95 ± 0.08	1.02 ± 0.09	1.00 ± 0.06
Hydrogenperoxide	0.85–17	1.00 ± 0.13	0.95 ± 0.12	1.10 ± 0.20
Hydroxyurea	760–7600	1.12 ± 0.08	0[a]	0[a]
Methylnitrosourea	25–100	1.09 ± 0.13	1.00 ± 0.13	1.00 ± 0.15
Novobiocin	125–450	1.10 ± 0.18	1.29 ± 0.08[b]	0.74 ± 0.13[b]
Hyperthermia	see below	0.85 ± 0.14	1.30 ± 0.09[b]	0.81 ± 0.10[b]
UV-Light	see below	1.00 ± 0.10	1.05 ± 0.15	0.97 ± 0.07
X-rays	see below	1.40 ± 0.18[b]	1.31 ± 0.12[b]	1.30 ± 0.15[b]

Hyperthermia: 30 min at 43 and 45 °C, UV-Light: 16–128 $J\,m^{-2}$, X-rays: 4–32 Gy; [a] Hydroxyurea without significant effects on SED and VISC, [b] $p < 0.05$ (student's t-test).

Table II. Nucleoid sedimentation (SED) and viscosity of alkaline lysates (VISC) of chicken embryo brain cells immediately following *in vitro*-exposure to the agents listed below. Pre-irradiation: Exposure of the embryos to 10 cGy at the 14th day of development, *i.e.* 24 h before cell preparation.

Agent	SED [cm]		VISC [%]	
	No pre-irradiation	Pre-irradiation	No pre-irradiation	Pre-irradiation
	1.81 ± 0.17	1.90 ± 0.15	100 ± 15	95 ± 18
Ethidiumbromide				
2.0 µg/ml	1.98 ± 0.25	1.86 ± 0.15	131 ± 12	136 ± 10
25.0 µg/ml	4.11 ± 0.22	4.70 ± 0.34	113 ± 12	110 ± 13
200.0 µg/ml	2.99 ± 0.22	4.13 ± 0.41[a]	56 ± 7	35 ± 10[a]
Novobiocin				
180 µg/ml	1.50 ± 0.15	1.88 ± 0.18[a]	167 ± 13	144 ± 11[a]
900 µg/ml	2.10 ± 0.21	2.22 ± 0.19	104 ± 8	109 ± 11
1800 µg/ml	6.20 ± 0.41	6.15 ± 0.37	63 ± 15	59 ± 10
Hyperthermia				
30 min/43 °C	2.31 ± 0.30	3.01 ± 0.18[a]	75 ± 7	61 ± 9[a]
30 min/45 °C	4.13 ± 0.38	5.29 ± 0.48[a]	68 ± 7	56 ± 6[a]

[a] $p < 0.05$ (student's t-test).

400 µg/ml (Table III). At lower EB-concentrations, no significant differences existed between both cell species with regard to the radioadaptive response (results presently not given in detail). Apart from this, there existed remarkable cellular peculiarities: Whereas in liver-cells, nucleoid sedimentation increased in dependence on the EB-concentration, the EB-induced elevation of the nucleoid's sedimentation distance in brain-cells exhibited two distinctly different maxima at about 25 and $\gtrsim$ 400 µg EB per ml (Table III).

As evidenced by previous investigations (Tempel and Schleifer, 1995), the adaptive response of the chicken embryo cells is reflected, inter alia, by a decrease in the sensitivity of scheduled DNA synthesis to "challenge doses" (4–32 Gy) of X-rays, priming doses exhibiting an optimal effect being 10–20 cGy. Therefore, pre-irradiated and

Table III. Nucleoid sedimentation of chicken embryo brain- and liver-cells immediately following *in vitro*-exposure to ethidium bromide. NP: No adapting pre-irradiation, P: Adapting pre-irradiation with 10 cGy at the 14th day of development, *i.e.* 24 h before cell preparation.

Ethidiumbromide [µg/ml]	0	25	50	100	150	200	400
Brain Cells							
NP	1.81 ± 0.17	4.11 ± 0.20	3.45 ± 0.35	2.26 ± 0.36	2.70 ± 0.30	2.99 ± 0.22	3.85 ± 0.65
P	1.90 ± 0.15	4.70 ± 0.34	4.07 ± 0.37	3.12[a] ± 0.38	3.80[a] ± 0.46	4.25[a] ± 0.55	4.62 ± 0.37
P/NP	1.05	1.14	1.18	1.38	1.41	1.42	1.20
Liver Cells							
NP	2.63 ± 0.14	3.51 ± 0.39	3.90 ± 0.35	4.68 ± 0.29	5.10 ± 0.40	6.51 ± 0.31	[b]
P	2.68 ± 0.25	4.20 ± 0.40	5.05[a] ± 0.22	6.28[a] ± 0.29	6.45[a] ± 0.25	6.49 ± 0.28	[b]
P/NP	1.02	1.20	1.29	1.34	1.26	1.00	–

[a] $p < 0.05$ (student's t-test); [b] nucleoids at the bottom of the tubes; the true sedimentation distance could not be quantified.

Table IV. Nucleoid sedimentation (SED) and scheduled DNA synthesis (SDS) of chicken embryo brain cells in dependence on the adapting (= priming) dose of X-irradiation. The sedimentation analyses were performed in cells immediately following a 30 min pre-incubation with ethidium bromide (200 μg/ml). The values listed below represent the mean of the ratios (Q) of adapted to non-adapted cells.

Dose of X-rays [cGy]	Q Adapted/non-adapted cells SED	SDS
–	1.00 ± 0.05	1.00 ± 0.12
5	1.09 ± 0.12	1.16 ± 0.12
10	1.24 ± 0.07	1.36 ± 0.17[a]
20	1.09 ± 0.08	1.20 ± 0.09
40	1.00 ± 0.08	0.90 ± 0.14
80	0.92 ± 0.10	0.86 ± 0.19

[a] $p < 0.05$ (student's t-test).

non-pre-irradiated brain cells were compared with respect to the adapting doses of X-irradiation by the nucleoid sedimentation technique and by estimating the inhibition of schedule DNA synthesis *in vitro* following a challenge dose of 16 Gy. Comprising a priming dose-range of 5–40 cGy, the results given by Table IV show a rather close correlation between both parameters, *i. e.,* the increase in the nucleoid sedimentation distance in EB-treated brain cells and the decrease in the sensitivity of scheduled DNA synthesis to X-irradiation *in vitro.*

In a last series of experiments, DNA was highly purified from primed and non-primed brain cells and exposed to DNase I either without additional agents or in the presence of some DNase I-activity inhibiting agents (Link and Tempel, 1991). As shown by the results summarized in Table V, no significant differences could be seen between both cell species. Therefore, under the conditions used, neither the sensitivity of DNA digestion by DNase I nor the DNA accessibility to various DNA-interactive agents seem to be changed in radioadapted cells.

Like polyanions in general, nucleic acids are non-specifically bound to the *AT protein* which interferes with its access to the alkylated DNA base, *i. e.,* O^6-alkylguanine, thus inhibiting AT activity (Bhattacharyya *et al.,* 1990). Therefore, the inhibition of the AT activity by highly purified DNA from non-adapted and adapted chicken embryo brain cells was tested, DNA concentrations ranging between 6.25 and 100 μg/ml. The DI_{50}-values (DNA concentrations inhibiting AT activity by 50%), as determined from the dose-effect-curves, were 20.3 ± 1.5 μg/ml in non-adapted and 20.5 ± 1.8 μg/ml in pre-irradiated cells. Thus, neither the DNase I assay nor the AT test revealed significant differences between both cell species.

Discussion

Support of the existence of an radioadaptive response in the chicken embryo has been provided by previous investigations (Tempel and Schleifer,

Table V. DNase I activity with highly purified DNA from chicken embryo brain cells (1.40 ± 0.24 mg DNA/brain). Pre-irradiation: Exposure of the embryos to 10 cGy at the 14th day of development, *i. e.* 24 h before cell- and DNA-preparation.

DNase I Activity	No pre-irradiation	Pre-irradiation
V (Increase in absorbance at 260 nm/20 min)	0.2907 ± 0.0650	0.3058 ± 0.0509
Apparent K_M [μg/ml]	242	243
DI_{50}-values of inhibiting substances[a] [μg/ml]		
Actinomycin D	20.0 ± 5.1	20.0 ± 4.8
Doxorubicin	12.5 ± 1.2	12.4 ± 1.3
Ethidiumbromid	8.0 ± 0.9	8.1 ± 1.0
Hoechst 33258[b]	12.0 ± 1.1	11.8 ± 1.2
Methylnitrosourea	124.0 ± 0.1	123.9 ± 0.12
Novobiocin	42.0 ± 5.0	40.0 ± 6.2

[a] DI_{50} = Concentrations inhibiting DNase I activity by 50 per cent;
[b] Hoechst 33258: 2′-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1 H-benzimidazole (Trihydrochloride) (Sigma No. B 2883).

1995). Whereas the specificity of the priming agent has to be investigated in the future, the results presented in Table I suggest that – with regard to the challenge agents used and within the limits of the techniques available in the present study – the primed response seems to be rather specific in the chicken embryo. As to the mechanism of the priming reaction, radioadaptation may be the result of an increase in DNA repair and/or specific DNA repair enzymes (see, *e.g.,* Joiner, 1994; Shadley, 1994; Wojewodzka *et al.,* 1994). Previous results, however, allowed the conclusion that a decrease in the initial radiation damage may be the dominant mechanism of the priming reaction of the chicken embryo (Tempel and Schleifer, 1995).

As suggested by an increasing number of investigations, nuclear and chromatin structures are highly implicated in the cellular radiation sensitivity (Olive, 1992; Schwartz and Vaughan, 1993; McMillan and Peacock, 1994; Oleinick and Chiu, 1994; Nygren *et al.,* 1995), *e.g.,* DNA conformation and higher-order chromatin structure (Oleinick and Chiu, 1994; Nygren *et al.,* 1995), nuclear proteins such as histones (Olive and Banáth, 1995), and DNA-nuclear matrix interactions (Schwartz and Vaughan, 1993; Malyapa *et al.,* 1994) being molecular determinants of DNA damage and DNA repair and, therefore, of cellular radiosensitivity (McMillan and Peacock, 1994). One means for studying alterations in higher-order DNA structure is the nucleoid sedimentation technique (Cook and Brazell, 1975, 1976). With regard to adaptive responses, Wojewodzka *et. al.* treated human lymphocytes with hydrogen peroxide (10 μM) or with 1 cGy X-rays. An about 30% decrease in the frequency of micronuclei upon subsequent X-irradiation (1.5 Gy) and an increase in the nucleoid sedimentation distance by 10%, when measured 90 min after the adaptive treatment and preceding the challenge dose, has been observed. Sedimentation of nucleoids at ethidium bromide (EB) concentrations within the range of ≤ 6 μg/ml was carried out, the dye was included in the sucrose gradient ("EB-titration"). By means of this technique, changes in loop size as well as alterations of the supercoiled density of the nucleoids of adapted lymphocytes could be excluded (Wojewodzka *et al.,* 1994). In the present investigations, DNA supercoiling was studied by the nucleoid sedimentation technique as well including "EB-titration". Confirming previous results (Tempel and Schleifer, 1995), nucleoid sedimentation was enhanced by 20–40% at EB concentrations of 50–400 μg/ml in brain- and liver-cell preparations following the adaptive pre-exposure *in ovo* by 10 cGy (Tables II and III). It is suggested, therefore, that the radioadapted chicken embryo cells exhibit a higher tendency to undergo positive DNA supercoiling (Cook and Brazell, 1975) and/or a greater stability of the DNA loop domains as evidenced, recently, by the "nucleoid fluorescent halo assay" in a radioresistant Chinese Hamster cell line (Malyapa *et al.,* 1994).

An increase in the nucleoid sedimentation distance was seen as well in radioadapted chicken embryo brain cells following exposure to hyperthermia and novobiocin (Tables I and II).

As shown by a number of investigations (see, *e.g.,* Roti Roti and Turkel, 1994; Stege *et al.,* 1995) *hyperthermia* induces intranuclear protein aggregation and affects DNA supercoiling. In general, the distance of nucleoid sedimentation increases as a function of exposure temperature and exposure time, and is proportional to an increased protein to DNA ratio in the nucleoids (ibid.). EB-induced changes in nucleoid sedimentation were investigated, *e.g.,* in mouse lymphoblastoma cells. Although exposure of the cells to hyperthermia did not alter the amount of DNA supercoiling, an increased ability of higher EB concentrations "to generate DNA with positive supercoiling" was observed and has been regarded to be "consistent with the ability of the L1210 DNA to approximate closed circular DNA" (Simpson *et al.,* 1987).

Novobiocin (NB) is a nonspecific inhibitor of DNA topoisomerase II (Kapiszewska and Lange, 1991). With cell-specific peculiarities, DNA relaxation, as reflected by a decrease in nucleoid sedimentation and an increase in the viscosity of alkaline cell lysates, is seen at rather low NB concentrations, whereas very high NB concentrations cause the contrary effect (Tempel, 1993; Table II). From the results presented in Table II the conclusion may be drawn that the adapting pre-irradiation inhibited or prevented the NB-induced DNA relaxation.

Whereas the nucleoid sedimentation technique and the viscometry of alkaline cell lysates revealed significant and rather challenge specific differences between radioadapted and non-adapted

cells (Tables I–IV), neither the sensitivity of highly purified DNA to digestion by DNase I nor the inhibition of DNase I activity by various DNA-interactive agents was changed in radioadapted cells. The same is true with respect to the inhibition of the enzyme O^6-alkylguanine-DNA alkyltransferase by the purified DNA. Within the limits of these enzyme assays, it follows therefore that the DNA structure itself remains unchanged in radioadapted chicken embryo cells. Taken together, the present results suggest an increase of tightening of protein-DNA interactions within the nuclear matrix (Cook and Brazell, 1976; Roti Roti and Painter, 1982) of radioadapted chicken embryo cells. The possibility, however, exists that the observed changes of nucleoid sedimentation could be secondary to additional binding of proteins usually associated with control nucleoid DNA (Simpson *et al.*, 1987) and/or proteins induced by the (priming) pre-irradiation (see, *e.g.*, Prasad *et al.*, 1995). With respect to the chicken embryo as a model for radioadaptive phenomena further investigations should be performed comprising the specificity of the priming agent and the possible reasons of the apparently high challenge specificity of radioadapted chicken embryo cells as suggested by the present studies.

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