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Hydrogen peroxide and sequence-specific DNA damage in human cells

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Abstract Exposure of HeLa cells in monolayer culture to increasing concentrations of exogenously added H_2O_2 causes damage to cellular DNA. When the DNA is subsequently isolated from the non-apoptotic cells remaining in such cultures, evidence was obtained to suggest that the DNA damage elicited in intact cells was non-random and that certain nucleotide sequences associated with, or related to, the genes for heat shock protein 60 and catalase were more susceptible to damage than others. In contrast, these particular sequences were not specifically susceptible to damage when naked human DNA was exposed directly to H_2O_2 in vitro. On an overall comparative basis, sequences in the genes encoding catalase, α-1 antitrypsin and β-actin appear more vulnerable to H_2O_2 in vivo, than sequences in *H-ras* and the p53 gene which seem surprisingly resistant.

Key words: Hydrogen peroxide; HeLa cells; DNA damage; Heat shock protein 60 gene; Catalase gene; β-Actin gene; α-1 Antitrypsin gene; Ras gene; p53 gene; Restriction fragment length polymorphisms

1. Introduction

Oxidant by-products of normal metabolism can cause significant damage to DNA (as well as protein and lipid). Whilst cellular antioxidant defenses exist, these are not perfect and the number of oxidative 'hits' per cell in humans has been estimated at 10000 per day. Although DNA repair enzymes remove most, but not all, of the lesions formed [1] in experimental animals oxidative lesions increase with age [1,2].

Endogenously generated oxidants that could lead to cellular DNA damage include superoxide radicals and H_2O_2. Whilst these are established products of the 'respiratory burst' when the plasma-membrane NADPH-oxidase of neutrophils and macrophages is activated, it is now clear that both superoxide and H_2O_2 are released from a variety of non-inflammatory human cells such as fibroblasts, endothelial cells, smooth muscle cells, colonic epithelial cells and osteocytes [3]. In addition superoxide is normally generated *intracellularly*. The major source of such intracellular generation appears to be mitochondria [4–7]. Superoxide arises from single electron transfer to oxygen but is probably converted to membrane permeable H_2O_2 by Mn-superoxide dismutase located in mitochondria.

There have been many reports to indicate that the human genome is potentially vulnerable to damage (or mutation) by H_2O_2 [8]. Important questions, however, relate not just to the chemical nature of the DNA damage [9,10] but also to the particular genomic sequences that are vulnerable. We have examined DNA from HeLa cells exposed in culture to varying concentrations of exogenously added H_2O_2. When particular sequences associated with, or related to, the human genes encoding heat shock protein 60 and catalase are probed, it appears that damage elicited in *intact* HeLa cells by H_2O_2 is non-random, with certain nucleotide sequences being more vulnerable than others. On an overall comparative basis, sequences in the genes encoding catalase, α-1 antitrypsin and β-actin appear more sensitive to H_2O_2 in vivo than sequences in *H-ras* or the p53 gene.

2. Materials and methods

2.1. Cell culture

HeLa cells were established as monolayer cultures at 37°C in Eagle's minimal essential medium supplemented with 10% (v/v) calf serum (Gibco BRL, Paisley, UK). Triplicate monolayer cultures (seeded at 0.5×10^6 cells per 3.5 cm petri dish) were first established by growth for 48 h before exposure to exogenously added H_2O_2. Growth was then normally allowed to continue for a further 20 h at which time cells that had detached from the culture dish were removed along with the medium. The cells that remained attached to the surface of the culture dishes were then used for the preparation of DNA as described below. The bulk of the cells that become detached from the culture dish at this stage after such treatment exhibit characteristics of apoptosis, i.e. condensation of chromatin around the inner nuclear membrane and extensive internucleosomal fragmentation [11,12].

2.2. DNA isolation

HeLa cells remaining attached to the monolayers were first washed with phosphate buffered saline (PBS) and then scraped off into 150 mM NaCl–10 mM Tris-HCl pH 7.5–10 mM EDTA at 10^6 cells per 6 ml. Sodium dodecyl sulfate (SDS) was added to 0.5% (w/v) and ribonuclease solution (previously heated to 100°C for 15 min) added to 100 μg/ml and the solution held at 37°C for 30 min. Proteinase K was then added to 100 μg/ml and incubation continued at 40°C for 60 min. An equal volume of water-saturated phenol was added and the mixture shaken at 4°C for 20 min. The phases were separated by centrifugation at $2000\times g$ for 10 min. The top layer was removed and extracted with chloroform/isoamylalcohol (24:1). Again the phases were separated by centrifugation. The resulting top layer was removed and mixed gently with 0.5 vol 7.5 M ammonium acetate. 2 vol ethanol was then added and mixed thoroughly. After 18 h at −20°C the DNA was collected by centrifugation at $2000\times g$ for 30 min. The pellet was washed briefly with 2.5 M ammonium acetate–66% (v/v) ethanol and then with 70% (v/v) ethanol. After air drying, the sticky DNA was dissolved in 10 mM Tris-HCl pH 7.5–10 mM EDTA.

For the isolation of DNA from human placenta, the tissue was first minced and then homogenised in ice-cold 100 mM NaCl–1 mM EDTA–50 mM Tris-HCl pH 7.5. SDS was then added to a final concentration of 0.5% (w/v). Thereafter the procedures followed were identical to those already described for the isolation of HeLa cell DNA (i.e. including the ribonuclease and proteinase K treatments).

2.3. Electrophoretic analysis of DNA

DNA samples from cells were analysed by electrophoresis through 1% agarose gels under neutral conditions. The DNA standards used for size comparisons were *Hae*III digests of ΦX174 phage DNA and *Hin*dIII digests of lambda phage DNA.

2.4. Restriction enzyme digestion of HeLa DNAs

HeLa cell DNA samples were digested with specific restriction en-

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donucleases according to manufacturer's directions at 2–4 units enzyme/μg DNA in a total volume of 1 μg/5 μl at 37°C for 18 h.

2.5. Gene sequence probes

The human heat shock protein 60 gene probe was a 1.5 kb *Pst*I fragment of the corresponding cDNA isolated from the plasmid pUCHS601 as supplied by StressGen Biotechnologies Corp. Victoria, BC, Canada [13]. The human catalase gene probe was a single copy cDNA obtained by screening a human cDNA library with an oligonucleotide corresponding to 17 bp of the human erythrocyte catalase. It was isolated as a 1 kb fragment cloned into the *Pst*I site of pKT218 [14]. The α-1 antitrypsin cDNA probe was also isolated as a 1 kb fragment cloned into the *Pst*I site of pKT218 (Boyd, P.A., unpublished). After digesting the above plasmids with *Pst*I, the resulting fragments were separated using 0.7% low melting point agarose and the required fragments recovered. The 1.8 kb human β-actin cDNA probe was from Clontech Laboratories, Palo Alto, USA and the 0.8 kb human *H-ras* cDNA and 1.3 kb human p53 cDNA were from Oncogene Science, Uniondale, USA. All DNA fragments for use as probes were labeled directly using random primer extension and [alpha-^{32}P]ATP [15].

2.6. Hybridisation of gene probes to restriction enzyme digests of HeLa cell DNA

4 μg amounts of restriction enzyme digested HeLa cell DNA were applied to neutral agarose gels and electrophoresed at 5 V/cm for 18 h. The DNA was transferred to nitrocellulose membranes by the method of Southern [16] and the filters baked for 2 h at 80°C. The membranes were prehybridised in 5×SSC [SSC; 0.15 M NaCl–0.015 M Na citrate], 4×Denhart's solution [Denhart's solution; 0.1 g polyvinylpyrrolidone, 0.1 g bovine serum albumin, 0.1 g Ficoll 400 plus water to 5 ml], 10% (w/v) dextran sulfate, 0.1% (w/v) sodium dodecyl sulfate (SDS), 0.1% sodium pyrophosphate, 100 μg/ml denatured salmon sperm DNA at 68°C for at least 1 h before adding the heat-denatured sequence probe (15–25×10^6 cpm) and hybridising at 69°C for at least 16 h. The membranes were washed twice in 2×SSC, 0.1% SDS, 0.1% sodium pyrophosphate at 68°C and finally in 0.5×SSC, 0.1% SDS, 0.1% sodium pyrophosphate at 68°C.

3. Results

HeLa cells in monolayer culture were initially exposed for 20 h at 37°C to increasing concentrations of exogenously added H_2O_2 and DNA isolated from the cells remaining attached to the monolayer. Fig. 1 shows that when this DNA was subjected to electrophoresis through 1% neutral agarose gels, signs of degradation were obvious in the DNAs from cells exposed to 1 and 10 mM H_2O_2. Importantly, however, there were no signs of internucleosomal DNA cleavage symptomatic of apoptosis. In contrast, however, when the DNA from cells that had become detached from the monolayers during these treatments was similarly analysed, extensive internucleosomal DNA cleavage was observed (see lanes 5′, 6′ and 7′). In further experiments the effects of adding o-phenanthroline along with the H_2O_2 were examined. Fig. 1 also shows that in the case of cells remaining attached to the monolayer, o-phenanthroline (100 μM) appeared to block the degradative effects of 10 mM H_2O_2.

In order to investigate further the nature of the DNA damage elicited by exposure of growing HeLa cells to H_2O_2, the DNA samples, isolated from cells that remained with the monolayers, were subsequently digested with various restriction nucleases. The DNA fragments resulting from such restriction digests were then analysed for their ability to hybridise to gene sequence probes specific for different human genes.

In the first instance gene sequences associated with the human gene encoding heat shock protein 60 (hsp60) [17] were examined. The DNA samples, isolated from the HeLa cells remaining attached to the culture dishes after exposure to H_2O_2 as shown in Fig. 1, were digested with *Msp*I, or with *Xba*I or *Hae*III. After electrophoretic separation of the resulting DNA fragments, these were blotted onto nitrocellulose membranes and their ability to hybridise to a labeled human hsp60 gene sequence probe assessed. In the case of the *Xba*I and *Hae*III digested DNAs the number of fragments hybridising to the hsp60 gene sequence probe was 13 and 10 respectively, irrespective of the concentration of H_2O_2 to which the cultured HeLa cells were originally exposed. In the case of HeLa monolayer cell DNA digested with *Msp*I, 15 fragments hybridising to the hsp60 probe were detectable in all DNA samples with the exception of DNA isolated from monolayer cells remaining after exposure to 10 mM H_2O_2 (Fig. 2). In this particular case a 15 kb fragment appears to absent. In addition several other *Msp*I fragments occur at notably reduced levels in that DNA [indicated by (−) in Fig. 2].

For the purposes of comparison, the DNAs from the H_2O_2 exposed monolayer cells as shown in Fig. 1 were next digested with *Pst*I and the resulting fragments tested for their ability to hybridise to a labelled sequence probe for the human catalase gene. Although 5 main hybridising fragments were observed (Fig. 3) the intensity of hybridisation was notably less in the DNAs from cells exposed to higher H_2O_2 concentrations. Moreover the fragments at 2.2 kb and 1.0 kb appeared to be missing from the DNA of HeLa cells originally exposed to only 100 μM H_2O_2. These fragments were also absent from the DNA of cells exposed to 1 mM and 10 mM H_2O_2. Additionally in the DNA from cells exposed to the latter concentration, an 8.6 kb fragment was also absent (Fig. 3).

In further comparisons, when HeLa DNA was digested

Fig. 1. Neutral agarose gel electrophoresis of DNA isolated from adherent HeLa cells after exposure of monolayer cultures to various concentrations of H_2O_2 at 37°C in normal growth medium for 20 h. Lane 1: DNA molecular weight markers of sizes (from top to bottom) 23130, 9476, 6557, 4631, 2322, 2027. Lane 2: DNA molecular weight values 1353, 1078, 872, 603, 280. Lane 3: DNA from adherent cells exposed to no added H_2O_2. Lane 4: DNA from adherent cells exposed to 10 μM H_2O_2. Lane 5: cells exposed to 100 μM H_2O_2. Lane 6: cells exposed to 1 mM H_2O_2. Lane 7: cells exposed to 10 mM H_2O_2. Lane 8, cells exposed to 10 mM H_2O_2 in the presence of 100 μM o-phenanthroline. Lane 9 is naked placenta DNA exposed in vitro to 10 mM H_2O_2 and Lane 10: naked placenta DNA exposed in vitro to 1 mM H_2O_2–1 mM $FeCl_3$–1 mM EDTA for 20 h at 37°C. Lane 5′, 6′ and 7′ are the DNAs isolated from the non-adherent cells collected after exposure to 100 μM, 1 mM and 10 mM respectively.

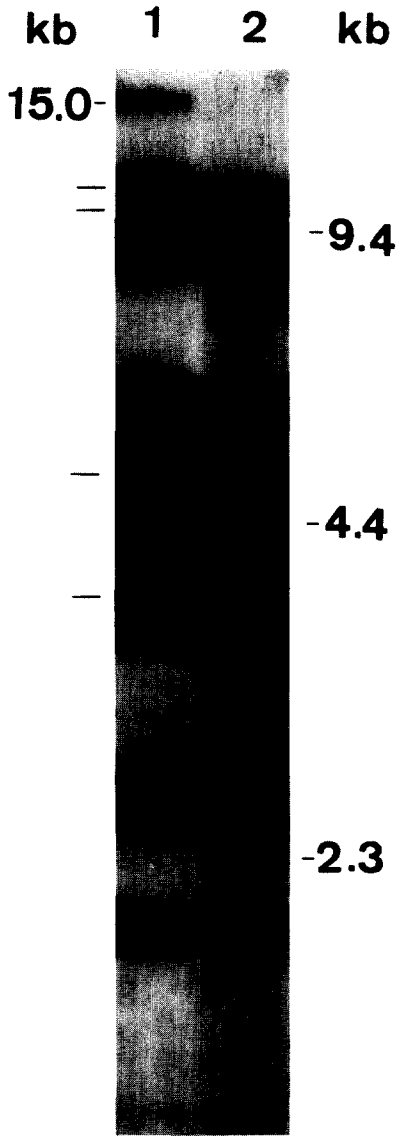

Fig. 2. Hybridisation of human hsp60 gene probe to *Msp*I digested DNA from HeLa cells (1), and from HeLa monolayer cells remaining after exposure in normal growth medium to 10 mM H_2O_2 for 20 h at 37°C (2). The numbers at the side indicate the size of, and migration of, specific fragments and marker DNAs in kb. *Msp*I fragments that occur at reduced levels in lane 2 are indicated with (−).

with *Msp*I, or *Eco*RI, a large number of fragments (7 and 10 respectively) hybridised with a β-actin cDNA probe. In contrast the DNAs from cells remaining attached after exposure to 1 mM or 10 mM H_2O_2, no fragments that hybridised with the actin probe remained. Similarly when *Msp*I digests were probed with a human α-1 antitrypsin cDNA probe, whilst two hybridising fragments were normally observed (3.2 kb and 3.9 kb) these were completely absent in DNA from cells exposed to 10 mM H_2O_2. A quite different picture emerged when the *Msp*I and *Eco*RI digests were probed with cDNA probes for the protooncogene *H-ras* [18] and the gene encoding the tumour suppressor protein p53 [19]. In Fig. 4A and B it can be seen that in the case of the p53 gene none of the hybridising fragments were lost even in cells exposed to 10 mM H_2O_2. A similar situation was observed with fragments hybridising to *H-ras* cDNA. In Fig. 4C the result of probing an *Xba*I digest with *H-ras* cDNA also shows no loss of hybridising fragments despite the concentration of H_2O_2 to which the cells were exposed (i.e. 100 μM–10 mM).

Since this apparent sequence-specific DNA damage was revealed in DNAs from intact cells previously exposed in culture to H_2O_2, comparative experiments were carried out in which naked human placenta DNA was directly exposed in vitro to varying concentrations of H_2O_2. When such naked placenta DNA was exposed directly to H_2O_2 (from 10 μM to 10 mM) no extensive degradation was observed when the treated DNA was analysed by direct electrophoresis through 1% neutral agarose gels (Fig. 1, lane 9). Moreover, when such in vitro treated naked DNA was digested with *Msp*I, or *Pst*I,

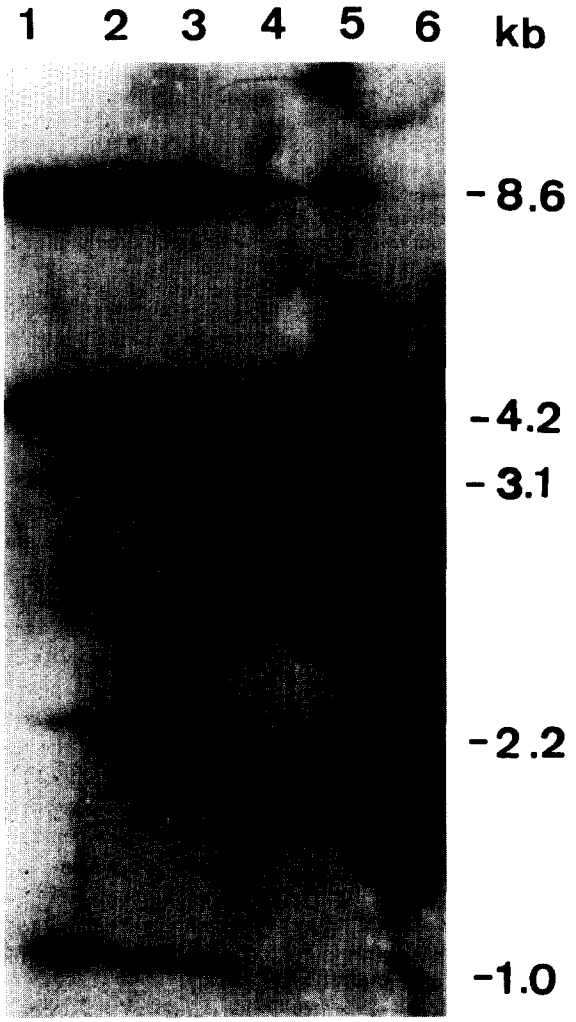

Fig. 3. Hybridisation of human catalase gene probe to *Pst*I digested DNA from HeLa monolayers remaining after exposure to varying concentrations of H_2O_2 in normal growth medium for 20 h at 37°C. Lane 1, no added H_2O_2 (control); lane 2, 1 mM H_2O_2; lane 3, 10 μM H_2O_2; lane 4, 100 μM H_2O_2; lane 5, 1 mM H_2O_2; lane 6, 10 mM H_2O_2. Numbers at sides indicate fragment sizes in kb.

and the resulting fragments probed with the hsp60 or catalase gene sequences, no changes were observed with regard to the number or level of hybridising fragments. In contrast degradation was observed when 1 mM H_2O_2 was added to the naked placenta DNA along with 1 mM EDTA and 1 mM $FeCl_3$ (Fig. 1, lane 10). When this partly degraded DNA was then digested with *Msp*I or *Pst*I and the fragments tested for their ability to hybridise to the gene probes, there was no selective loss of specific hybridising fragments. Instead there was an extensive loss of overall hybridisation to all fragments suggesting random, rather than any sequence-specific damage, under these conditions.

4. Discussion

Although the DNA isolated from HeLa cells that have been exposed to high concentrations of H_2O_2 (1 mM and 10 mM), yet remain attached to the culture dishes, shows evidence of degradation, this is not the internucleosomal fragmentation characteristic of cells undergoing apoptosis [10,11]. However, a proportion of HeLa cells, like BHK-21 cells [12], do undergo apoptosis when exposing to high concentrations of H_2O_2 (100 μM and above). Such apoptotic HeLa cells detach from the surface of the culture dish and can thereby be readily removed, thus providing a convenient opportunity to study DNA damage occurring with H_2O_2 treated HeLa cells remaining attached to the monolayer without the complication of apoptotic internucleosomal DNA cleavage.

H_2O_2 itself is not sufficiently reactive to attack DNA [8]. Indeed significant degradation was only observed when the naked placenta DNA was exposed to a H_2O_2–EDTA–$FeCl_3$ mixture which is known to generate hydroxyl radicals [20] which in turn can damage DNA nucleotides [10,21]. However, when the nature of this in vitro DNA damage was investigated in this study it appeared to be random rather than directed at any particular nucleotide sequence. Our observation that the cell permeable Fe-chelator o-phenanthroline [22] can nevertheless block DNA damage elicited in intact cultured HeLa cells following exposure of these cells to high concentrations of H_2O_2, supports the view that whatever the intracellular mechanisms that lead to the observed DNA damage in intact HeLa cells, they may well involve intracellular iron [8,23]. Such iron could be involved in the generation of damaging hydroxyl radicals [8,23].

When the location of damage elicited in monolayer cells following exposure to H_2O_2 was examined in relation to two human genes, for hsp60 and catalase, it was notable that the damage appeared non-random. The target sites for certain restriction enzymes such as *Msp*I in the case of hsp60 gene associated sequences, and *Pst*I in the case of sequences associated with the catalse gene, are differentially sensitive in as much as certain restriction fragments are lost or reduced depending on the concentration of H_2O_2 to which the cells were initially exposed. In contrast none of the target sequences for *Xba*I and *Hae*III in relation to the hsp60 gene seem especially sensitive. Overall it seems that targets in the hsp60 gene may be less vulnerable than those in the catalase gene. Additional experiments have suggested that other genes like catalase, such as those encoding actin or α-1 antitrypsin, were also notably vulnerable. This is in contrast to the genes encoding cell regulatory protein, Ras, and the tumour suppressor, p53, which seemed to be surprisingly resistant.

The reasons for the apparent differential sequence specificity of H_2O_2 elicited damage in intact cells may be complex.

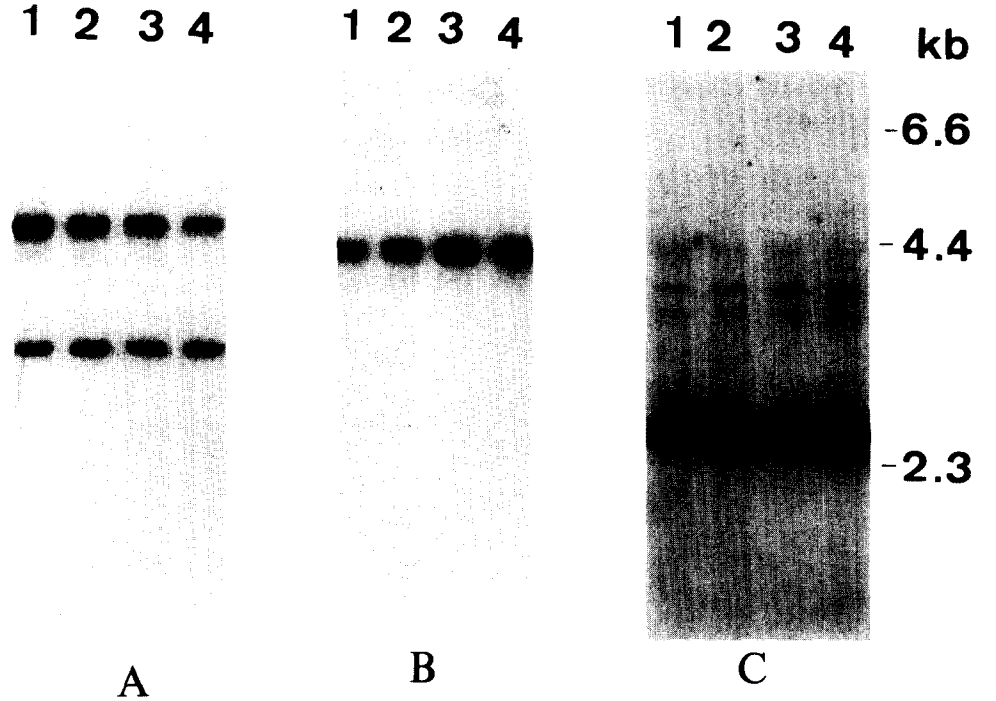

Fig. 4. Hybridisation of *H-ras* and p53 gene probes to restriction endonuclease digested DNA from HeLa cells monolayers remaining after exposure to varying concentrations of H_2O_2 in normal growth medium for 20 h at 37°C. (A) *Eco*RI digested DNAs probed with p53 gene cDNA; (B) *Msp*I digested DNAs probed with p53 gene cDNA; (C) *Xba*I digested DNAs probed with *H-ras* cDNA. Lanes 1, DNA from control HeLa cells; lanes 2, DNA from HeLa cells exposed to 100 μM H_2O_2; lanes 3, DNA from cells exposed to 1 mM H_2O_2; lanes 4, DNA from cells exposed to 10 mM H_2O_2. Number at side indicates the relative mobility of lambda phase fragments used as markers.

It is possible that the three dimensional arrangement of genes in interphase nuclei of intact HeLa cells is such that some genes and their associated sequences are simply more exposed than others by virtue of their nuclear location. Some genes of course may be more "protected" through their interaction with various chromosomal, or soluble cellular proteins. Alternatively it may be that the eventual intranuclear location of iron, capable of catalysing the formation of damaging hydroxyl radicals, is actually the most critical factor. This is because any hydroxyl radicals would be likely to only damage DNA structures in the immediate vicinity of the locus of their generation [21]. It should also be pointed out that Cu^{2+} could also catalyse the formation of hydroxyl radicals [10] and that copper has been detected specifically in chromatin structures [24]. Whatever the explanation, the finding that an oxidant which would be encountered by cells at loci of inflammation, or normally generated within most cells, can elicit sequence-specific DNA damage may have potential relevance to aging and age-related disease processes. On the other hand it may be significant that genes encoding certain critical cell regulatory molecules such as Ras and p53 seem less vulnerable to damage from such a natural oxidant.

It may be argued that we have used very high levels of exogenously added H_2O_2 in order to detect sequence-specific damage in cultured HeLa cells. However, in a previous report [12] we showed that due to catabolic effects, such high concentrations in cell growth medium are in fact extremely transitory. Even within 2–3 min of adding H_2O_2 at 10 mM to BHK-21, or HeLa cells, growing in normal serum supplemented growth medium at 37°C, the effective medium concentration of H_2O_2 drops to around 50 μM! By 20 h it is only approximately 0.5 μM. In short, extensive catabolism of H_2O_2 within the medium and within cells precludes any definitive statement about effective H_2O_2 concentrations other than the initial concentration added exogenously to the medium. Despite these arguments it is possible that the apparently sequence-specific DNA damage revealed in intact HeLa cells by our techniques, is in fact a manifestation of inefficient, or variable gene repair. The high concentrations of H_2O_2 could not only cause DNA damage but also either overwhelm or partly damage the repair systems themselves [25]. Another possibility, in view of observations indicating a link between repair of pyrimidine dimers and transcription [26] is that more actively transcribed sequences may be repaired more rapidly. However, it is premature to speculate on the basis of the few genes so far examined in HeLa cells without comprehensive data on their relative transcription rates under the conditions used. Moreover, in the case of "oxidative" DNA base damage, recent evidence indicates repair to occur as effectively in non-coding regions as in actively transcribed sequences [27].

References

[1] Ames, B.N., Shigenaga, M.K. and Hagen, T.M. (1991) Proc. Natl. Acad. Sci. USA 90, 7915–7922.
[2] Branda, R.F., Sullivan, L.M., O'Neill, J.P., Falta, M.T., Nicles, J.A., Hirsch, B., Vacek, P.M. and Albertina, R.J. (1993) Mutat. Res. 285, 267–279.
[3] Burdon, R.H. (1995) Free Radical Biol. Med. 18, 775–794.
[4] Loschen, G., Azzi, A. and Flohe, L. (1973) FEBS Lett. 33, 84–88.
[5] Boveris, A. and Cadenas, E. (1975) FEBS Lett. 54, 311–314.
[6] Nohl, H. and Heger, D. (1978) Eur. J. Biochem. 82, 563–567.
[7] Hennet, T., Richter, C. and Peterhaus, C. (1993) Biochem J. 289, 587–592.
[8] Meneghini, R. and Martins, E.L. (1993) in: DNA and Free Radicals (B. Halliwell and O.I. Aruoma, Eds.), Ellis Horwood, pp. 83–93.
[9] Dizdaroglu, M., Nackerdien, Z., Chao, B.-C., Gajewski, E. and Rao, G. (1991) Arch. Biochem. Biophys. 285, 388–390.
[10] Halliwell, B. and Aruoma, O.I. (1991) FEBS Lett. 281, 9–19.
[11] Korsmeyer, S.J. (1995) Trends Genet. 11, 101–105.
[12] Burdon, R.H., Gill, V. and Alliangana, D. (1995) Free Rad. Res., in press.
[13] Hickey, E., Brandon, S.E., Sadis, S., Small, G. and Weber, L.A. (1986) Gene 43, 147–154.
[14] Boyd, P., Van Heyningen, V., Seawright, A., Fekeke, G. and Hastie, N. (1986) Human Genet. 73, 171–174.
[15] Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13.
[16] Southern, E.M. (1975) J. Mol. Biol. 98, 503–517.
[17] Jindahl, S., Dudani, A.K., Singh, B., Harley, C.B. and Gupta, R.S. (1989) Mol. Cell Biol. 9, 2279–2283.
[18] Fasano, O., Taparowsky, E., Fiddes, J., Wiglar, M. and Goldfarb, M. (1953) J. Mol. Appl. Genet. 2, 173–180.
[19] Harlow, E., Williamson, N.M., Ralston, R., Helfman, D.M. and Adams, T.E. (1985) Mol. Cell Biol. 5, 1601–1610.
[20] Aruoma O.I., Halliwell, B., Gajewski, E. and Dizdaroglu, M. (1989) J. Biol. Chem. 264, 20509–20512.
[21] Dizdaroglu, M. (1993) in: DNA and Free Radicals (B. Halliwell and O.I. Aruoma, Eds.), Ellis Harwood, pp. 19–39.
[22] Mello Filho, A.C., Hoffmann, M.E. and Meneghini, R. (1984) Biochem. J. 218, 273–275.
[23] Saran, M. and Bors, W. (1989) Free Rad. Res. Commun. 7, 213–220.
[24] Lewis, C.D., Lebkrowski, J.S., Daly, A.K. and Laemmli, U.K. (1984) J. Cell Sci. Suppl. 1, 103–102.
[25] Enoch, T. and Norbury, C. (1995) Trends Biochem. Sci. 20, 426–430.
[26] Hanawalt, P.C. (1994) Science 266, 1957–1960.
[27] Bohr, V.A., Taffe, B.G. and Larminat, F. (1995) in: Oxidative Stress and Aging (R.G. Cutler, L. Packer, J. Bertram and A. Mori, Eds.), Birkauser-Verlag, Basel, pp. 101–110.