# Skin fibroblasts from spermine synthase-deficient hemizygous gyro male (*Gy*/Y) mice overproduce spermidine and exhibit increased resistance to oxidative stress but decreased resistance to UV irradiation

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Hemizygous gyro male (*Gy*/Y) mice are a model for X-linked hypophosphataemic rickets. As in humans, the disease is caused by deletions in the *Phex* gene, a phosphate-regulating gene having homologies with endopeptidases on the X chromosome. Some phenotypic abnormalities in *Gy*/Y mice have recently been attributed to the fact that the *Gy* deletion also includes the neighbouring spermine synthase gene, resulting in spermine deficiency. Spermine and its precursors spermidine and putrescine are essential for cell growth and differentiation. As a novel method for studying the function of spermine, we established primary cultures of skin fibroblasts from hemizygous *Gy*/Y mice. The *Gy*/Y cells contained no detectable spermine. In view of the fact that spermine is a free-radical scavenger *in vitro*, we were surprised to find that *Gy*/Y cells were more resistant to oxidative stress than their normal (X/Y) counterparts. However, our finding that spermidine accumulates markedly in the spermine-deficient *Gy*/Y cells can probably explain this increased resistance. It is the first indication that spermidine can serve as a free-radical scavenger *in vivo* and not only *in vitro*. When subjecting the *Gy*/Y cells to UV-C irradiation we made another interesting finding: the mutant cells were more sensitive than the normal X/Y cells. This finding indicates that spermine, probably because of its high-affinity binding to DNA, is important in protection against chromatin damage.

Key words: free radicals, ornithine decarboxylase, *Phex* gene, polyamines.

## INTRODUCTION

The polyamines putrescine, spermidine and spermine are essential for cell growth and differentiation. To maintain adequate concentrations of polyamines, cells depend on synthesis, degradation and transport, which are all regulated at many different levels [1]. Because of their polycationic nature and unique charge distribution, the polyamines are believed to be important for chromatin structure and stability [2]. Indeed, when cells are depleted of their polyamines by specific inhibitors of the polyamine biosynthetic enzymes, they exhibit increased sensitivity to DNA damage [3]. A role for the polyamines in transcriptional repression has recently been observed in yeast and might be due to their ability to stabilize highly condensed states of chromosomal fibres [4]. Polyamines have also been reported to be antioxidants [5] and to be important for DNA repair [6,7]. Spermine was recently shown to be a free-radical scavenger *in vitro* [8]. Ornithine decarboxylase (ODC) is one of the rate-limiting enzymes in the polyamine biosynthetic pathway. It is a highly regulated enzyme that catalyses the conversion of ornithine into putrescine, which is the first step in the synthesis of polyamines. Inhibition of ODC activity leads to reduced polyamine levels and cell cycle arrest [9], whereas overexpression may cause neoplastic transformation [10,11]. The polyamines themselves feedback regulate ODC at the level of translation [12], and by inducing the synthesis of antizyme which promotes degradation of ODC by the 26 S proteasome [13,14]. ODC may be regarded as a stress protein [15] and is induced by UV irradiation [16–19] and by exposure to reactive oxygen species [20,21]. Since the products of ODC activity are polyamines, it is conceivable that the production of polyamines upon ODC induction serves to protect cells against DNA lesions and free radical damage. The hemizygous gyro male (*Gy*/Y) mouse has served as a model for X-linked hypophosphataemia in humans [22]. The disease is due to a major deletion in the 5′ end of the mouse homologue of the human *PHEX* (phosphate-regulating gene having homologies with endopeptidases on the X chromosome; previously called *PEX*) gene, which encodes a neutral endopeptidase with significant homology to members of the membrane-bound metalloendopeptidase family [23]. The mutant mice have rickets/osteomalacia, reduced viability and a circling behaviour. The *Hyp* mouse, which likewise has a deletion in the *Phex* gene, exhibits a less complex phenotype than does the *Gy*/Y mouse [23,24]. This appears to be due to the fact that the *Gy*/Y mouse carries a more extensive deletion, including the neighbouring spermine synthase gene [25–27]. The latter is located about 39 kb upstream of the *Phex* gene. This deletion results in barely detectable levels of spermine, and elevated spermidine levels in all tissues examined. The lack of spermine is thought to explain the additional symptoms of the gyro phenotype [26,27]. The *Gy*/Y mouse represents the first vertebrate model for a major deficiency in the polyamine biosynthetic pathway. In the present study we have isolated primary fibroblasts from two male mice with the same genetic background; a *Gy*/Y mouse and a normal

Abbreviations used: BESPD, $N^1$,$N^8$-bisethylspermidine; DFMO, α-difluoromethylornithine; *Gy*/Y, hemizygous gyro male; ODC, ornithine decarboxylase; *Phex*, phosphate-regulating gene having homologies with endopeptidases on the X chromosome; RT–PCR, reverse-transcriptase-mediated PCR; SSAT, spermidine/spermine $N^1$-acetyltransferase; X/Y, normal littermate.

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X/Y littermate. Using the mutant cells, which proved to com pletely lack spermine, we have analysed whether spermine is important for their protection against cytotoxic and genotoxic exposure.

## MATERIALS AND METHODS

### Mice

Normal males (X/Y) and heterozygous gyro females (*Gy*/X) of the B6C3H mouse strain were obtained from the Jackson Laboratory (Bar Harbor, ME, U.S.A.). The *Gy* mutation is dominantly inherited and is X-linked [22]. *Gy* males are sterile and exhibit a more severe phenotype than female *Gy*/X mice.

### Cell culture

Skin biopsies were taken from two 3-week-old male littermates, one without phenotypic disturbances (X/Y), the other exhibiting gyro (*Gy*/Y) characteristics: smaller size, rickets and circling behaviour [22]. Cells that grew out of the biopsies were trypsin-treated and plated. The resulting monodisperse cultures were propagated until there was no evidence of cell types other than fibroblasts (five passages). The cells were grown in F12/Dulbecco's minimal essential medium containing 20 % (v/v) fetal calf serum, 0.24 % insulin/transferrin/selenium solution (Life Technologies) and 1 % (v/v) penicillin/streptomycin/fungizone solution (Life Technologies). Cells from the same passage were used for each individual experiment. Rat UMR-106 osteoblastic cells were grown in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) fetal calf serum.

### Reverse-transcriptase-mediated PCR (RT–PCR) analysis

Total RNA was extracted from *Gy*/Y, X/Y and UMR-106 cells by the guanidinium isothiocyanate method [28]. RT–PCR was performed with Ready-To-Go RT–PCR beads (Pharmacia). The primer pairs used were: CCAGCGAAGAACTCATACAGC (sense) and AATTCTTGGTCAGCACCACC (anti-sense) for collagen I a2 mRNA, CCACACTATGGCAGCAGC (sense) and CAGCACACGTCATTCTCATC (anti-sense) for spermine synthase mRNA, CAGAATGCATAGAAGCCGC (sense) and TGGATGCCTTGTCATCAGG (anti-sense) for *Phex* mRNA, and GTGACAGAGGAGCCGACGTC (sense) and GGTTT-GAGCCAGGGGAGGTG (anti-sense) for antizyme mRNA. The RT–PCR products were separated by electrophoresis in a 1 % (w/v) agarose gel.

### Polyamine analysis

The reversed-phase HPLC method developed by Seiler and Knödgen [29] was used to quantify the cellular polyamine content. Cell pellets were sonicated in 0.2 M $HClO_4$ and centrifuged at 20000 ***g*** (10 min, 4 °C). The supernatant was analysed with a Varian Vista 5500 liquid-chromatography system equipped with a Model 9090 AutoSampler, a Model 2010 HPLC Pump, a Model 2050 variable-wavelength UV detector, and a Fluorichrom fluorescence detector. The software Dynamax HPLC Method Manager and MacIntegrator (Rainin Instrument Company) were used together with a Macintosh SE/30 for method editing, HPLC control, and data collection and analysis (peak identification and quantification).

### Determination of GSH

For the determination of GSH, the HPLC method described by Anderson [30] was used. In brief, GSH in a sample was reacted with monobromobimane to form a fluorescent adduct that was analysed with the use of the HPLC apparatus described above. To obtain samples, cells in a confluent 100 mm culture dish were lysed by the addition of 3.33 % (w/v) 5-sulphosalicylic acid/0.16 mM EDTA solution. The lysate was centrifuged for 5 min at 10000 ***g*** and the supernatant was collected for HPLC analysis. The pelleted cellular debris was sonicated in PBS and used for protein determination with the Bio-Rad protein assay.

### Analysis of ODC activity

ODC activity was measured as described by Jänne and Williams-Ashman [31]. Cell pellets were sonicated in assay buffer [0.1 M Tris/HCl (pH 7.5)/0.1 mM EDTA/12.5 mM dithiothreitol] containing 1 % (v/v) Triton X-100. After centrifugation at 10000 ***g*** for 3 min, an aliquot of each supernatant was mixed with [$^{14}$C]ornithine and pyridoxal 5′-phosphate. $^{14}CO_2$ released in the catalytic reaction was trapped in hyamine hydroxide and the radioactivity was determined in a Beckman LS6500 scintillation counter. One unit was defined as 1 nmol of $CO_2$ released/h. Because the cellular ODC activity varied somewhat between passages, we always used cells from the same passage for each individual experiment.

### Northern blot analysis

Total RNA was extracted from cells by the guanidinium isothiocyanate method [28]. The RNA was separated electrophoretically in a formaldehyde-containing 1 % (w/v) agarose gel and subsequently transferred to a Hybond-N$^+$ membrane (Amersham) by vacuum blotting. The RNA was hybridized to a [$^{32}$P]dCTP random primer-labelled mouse ODC-antizyme cDNA [32].

### Analysis of spermidine/spermine N$^1$-acetyltransferase (SSAT) activity

SSAT activity was determined as described by Della Ragione and Pegg [33]. To induce SSAT, 100 $\mu$M $N^1$,$N^8$-bisethylspermidine (BESPD) was added to the growth medium. At various times after the addition, the cells were trypsin-treated, pelleted by centrifugation and lysed by the addition of 1 % (v/v) Triton X-100 (in reaction buffer, which was 10 mM Tris/HCl, pH 7.8). After centrifugation at 10000 ***g*** for 3 min, an aliquot of each supernatant was mixed with spermidine (3 mM final concentration) and [1-$^{14}$C]acetyl-CoA (0.4 $\mu$Ci/ml, final concentration). After incubation at 37 °C for 1 h, the reaction was stopped by addition of 0.2 vol. of 1 M hydroxylamine hydrochloride. The reaction mixture was boiled for 3 min and cleared by centrifugation at 3000 ***g*** for 5 min. An aliquot was then applied to a cellulose phosphate paper (Whatman P81) disc. After the disc had been washed with water and ethanol on a sintered-glass funnel, it was dried and the radioactivity was determined in a Beckman LS6500 scintillation counter. One unit was defined as 1 nmol of acetylspermidine formed/h.

### Exposure to $H_2O_2$

Cells were seeded at a density of 10000 cells/ml and grown for 24 h. The medium was then replaced with fresh medium containing 250 $\mu$M $H_2O_2$. After 24 h the cells were harvested and counted in a haemocytometer. To estimate cell survival, the number of viable cells in $H_2O_2$-exposed cultures was compared with that in unexposed cultures. The cells were pelleted by centrifugation and analysed for ODC activity.

### Irradiation with UV-C

Cells were seeded at a density of 10000 cells/ml and grown for 24 h. To remove UV-absorbing components of the medium, the cells were washed with PBS. After removal of the lid and the PBS, the cell layer was subjected to UV-C irradiation (254 nm) with a Stratalinker 1800 equipped with an internal dose controller set at 20 or 100 J/m². Fresh medium was added and cell survival was determined after 24 h as described above. The cells were pelleted and analysed for ODC activity.

## RESULTS

### Characterization of skin fibroblast cultures established from *Gy*/Y mice and their normal X/Y littermates

The gene deletion responsible for the gyro phenotype [22] includes not only the spermine synthase gene but also the *Phex* gene, which is expressed in calcified tissues (bone and teeth) of normal mice. We therefore chose to isolate skin fibroblasts, which exhibit no significant *Phex* gene expression [24]. X/Y littermates were used as controls. Other possible controls would have been cells from *Gy*/X (female) or male *Hyp* mice. However, these would have had the disadvantage of representing a different sex and a different genetic background respectively. A fibroblast marker (collagen I a2) was used in an RT–PCR experiment to make sure that the cultures established from skin of *Gy*/Y mice and their X/Y littermates were indeed fibroblastic (Figure 1). With the same method we demonstrated the presence of spermine synthase mRNA in X/Y cells but not in *Gy*/Y cells (Figure 1). In agreement with the results of others [24], the *Phex* gene was found not to be expressed in fibroblasts (*Gy*/Y and X/Y cells) but to be expressed in osteoblasts (UMR-106 cells) (Figure 1).

### Effects of polyamine depletion on the growth of *Gy*/Y cells in comparison with their normal counterpart

Under standard conditions, the *Gy*/Y and X/Y cells exhibited similar growth patterns. Figure 2 shows representative growth curves, indicating generation times of approx. 2 days for both cell lines. Despite their similar growth rates, they responded differently to treatment with 5 mM $\alpha$-difluoromethylornithine (DFMO) [34], an enzyme-activated irreversible inhibitor of ODC. Thus *Gy*/Y cells ceased to proliferate, whereas normal X/Y cells continued to grow, although at a 50% decreased rate. In both cell lines, treatment with DFMO eradicated the ODC activity (see Figure 4A) and as a result decreased the putrescine content to an undetectable level (Figure 3A). In the X/Y cells the spermidine level also decreased precipitously, to a very low level (Figure 3B). Because untreated *Gy*/Y cells had 4-fold more spermidine than did X/Y cells, treatment with DFMO only caused a decrease in spermidine content to a level corresponding to that of untreated X/Y cells (Figure 3B). In X/Y cells, treatment with DFMO exerted no major effect on the spermine level. This is probably why they were able to continue their growth, albeit at a lower rate. Although the *Gy*/Y cells had no spermine (Figure 3C) because of the deletion in their spermine

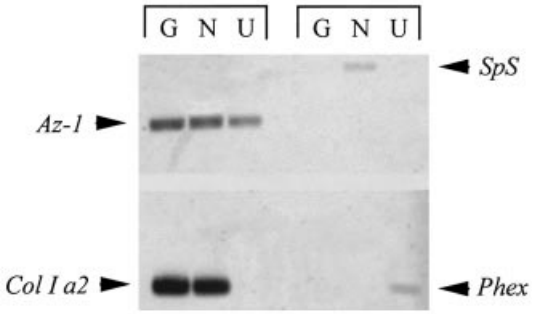


**Figure 1 Characterization of the mutant *Gy*/Y cells (G) and their normal X/Y counterparts (N) by RT–PCR**

The UMR-106 osteoblastic cell line (U) was used as a positive control for *Phex* expression. Total RNA was extracted, of which 0.6 $\mu$g was used for the RT–PCR together with sense and antisense primer pairs specific for the following transcripts: antizyme (*Az-1*, a positive control), spermine synthase (*SpS*), collagen I a2 (*Coll I a2*, a fibroblast marker), and *Phex*.

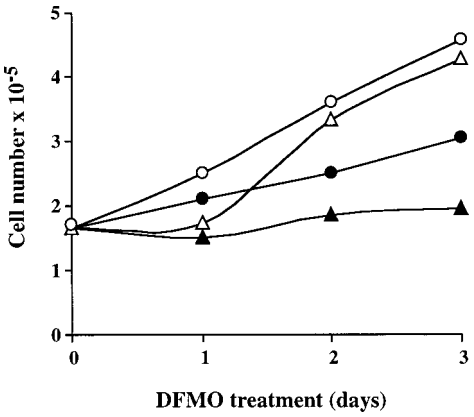


**Figure 2 Growth of *Gy*/Y cells (△,▲) and their normal X/Y counterparts (○,●) in culture, and antiproliferative effects of DFMO-mediated polyamine depletion**

The cells were grown in the absence (△,○) or presence (▲,●) of 5 mM DFMO.

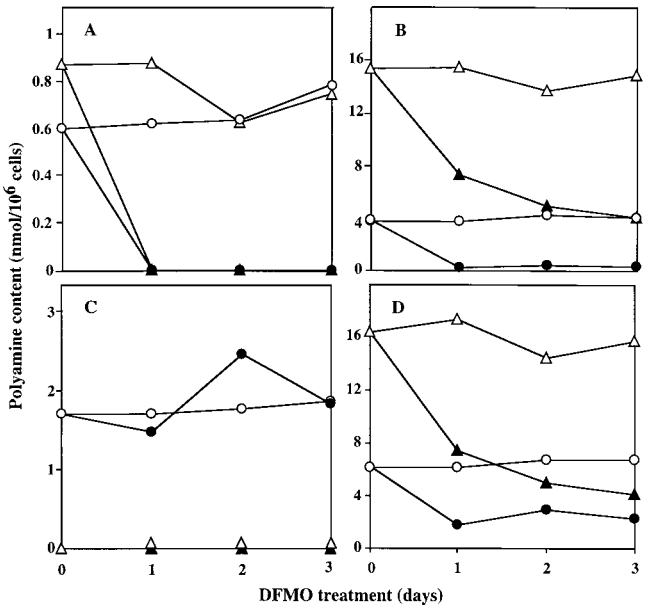


**Figure 3 Effects of treatment with DFMO on the polyamine content of *Gy*/Ycells (△,▲) in comparison with normal X/Y fibroblasts (○,●)**

The cells were grown in the absence (△,○) or presence (▲,●) of 5 mM DFMO, and were assayed for their putrescine (**A**), spermidine (**B**) and spermine (**C**) content by reversed-phase HPLC. The values in (**A**–**C**) were used to calculate the total polyamine content (**D**).

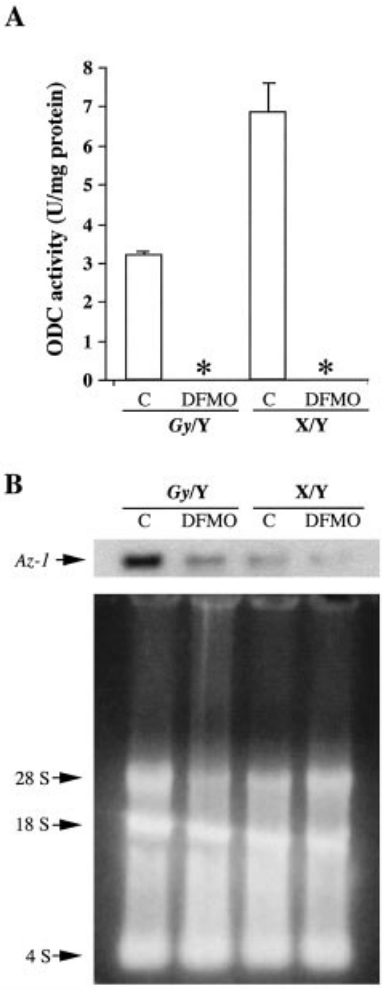


**Figure 4 ODC activity and antizyme (*Az-1*) mRNA level in *Gy*/Y cells in comparison with normal X/Y cells**

(**A**) Cells were cultured for 1 day before being harvested. Results are means ± S.D. ($n = 3$). An asterisk indicates that the ODC activity was undetectable. (**B**) Northern blot analysis of cells cultured for 2 days in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 5 mM DFMO. Total RNA was extracted, of which 10 $\mu$g was separated by agarose-gel electrophoresis. After transfer of the RNA to a membrane, hybridization with an antizyme probe and subsequent autoradiography was performed (top panel). Equal loading was verified by photographing the ethidium-bromide-stained RNA gel (bottom panel). The autoradiogram and the photograph were scanned with an Agfa SnapScan 1236, and processed with Adobe Photoshop 5.0 (FotoLook plug-in).

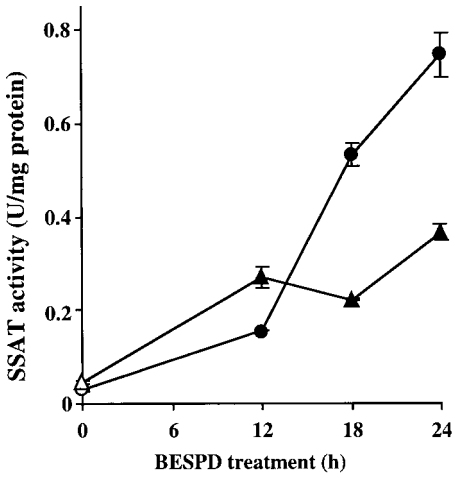


**Figure 5 Induction of SSAT by BESPD in *Gy*/Y cells (▲) and in their normal X/Y counterparts (●)**

Untreated cells (△,○) and cells treated with BESPD (100 $\mu$M) were harvested at the time points indicated, then assayed for SSAT activity as described in the Materials and methods section. Results are means ± S.D. ($n = 3$).

synthase gene, the compensatory increase in spermidine level was so marked that their total polyamine content was almost 3-fold greater than that of X/Y cells (Figure 3D). Nevertheless, treatment with DFMO had a stronger antiproliferative effect on *Gy*/Y cells (Figure 2), indicating that maintenance of the spermine level is more advantageous for growth than is maintenance of the spermidine level.

### ODC activity, antizyme expression and SSAT activity in *Gy*/Y cells in comparison with their normal counterpart

The polyamines are known to exert feedback regulation of the activity of ODC [1]. It was therefore of interest to know how the lack of spermine and the excessive increase in spermidine would affect the ODC activity. Figure 4(A) shows that the ODC activity of *Gy*/Y cells was markedly suppressed in comparison with the normal (X/Y) counterpart. Thus, even though *Gy*/Y cells have no spermine, the accumulation of spermidine was sufficient to affect their ODC activity adversely. This finding is at variance with that of Lorenz et al. [26], who found that the ODC activity was elevated in all tissues analysed from *Gy*/Y mice. The difference is probably due to the fact that the spermidine concentration in the *Gy*/Y cells by far exceeded those in *Gy*/Y mouse tissues. In fact, the total polyamine content of the *Gy*/Y cells was almost 3-fold that of normal X/Y cells (Figure 3D), whereas the total polyamine content of *Gy*/Y mouse tissues was not even twice that of normal mouse tissues [27]. There are several possible methods by which spermidine can down-regulate ODC, for example by interfering with ODC mRNA translation [12] or by inducing ribosomal frameshifting of the antizyme mRNA [35], leading to the synthesis of antizyme, which promotes the degradation of ODC by the 26 S proteasome [14]. In a previous study we demonstrated that spermidine depletion causes decreased transcription of the antizyme gene [32]. Analogously, the present study shows that *Gy*/Y cells with their elevated spermidine level have a much higher steady-state level of antizyme mRNA than their normal counterpart (Figure 4B). Moreover, when the spermidine content of the *Gy*/Y cells was decreased to a normal level by treatment with DFMO (Figure 3B), the amount of antizyme mRNA decreased to a level comparable with that of untreated X/Y cells (Figure 4B). Because spermidine is known to stimulate polyamine catabolism by inducing SSAT activity [36], we analysed *Gy*/Y and X/Y cells for their activity of this enzyme. Surprisingly, the SSAT activity was as low in the *Gy*/Y cells as in the X/Y cells. Interestingly, when measuring the ability to induce SSAT, by treatment with the polyamine analogue BESPD, we found a greater induction in X/Y cells than in *Gy*/Y cells (Figure 5). Many polyamine analogues induce SSAT by either increasing the transcription of the gene or by changing enzyme stability [36,37]. Taken together, these findings suggest that the *Gy*/Y cells are more resistant to the induction of polyamine catabolism than are the X/Y cells and might explain why the *Gy*/Y cells can maintain their high level of spermidine.

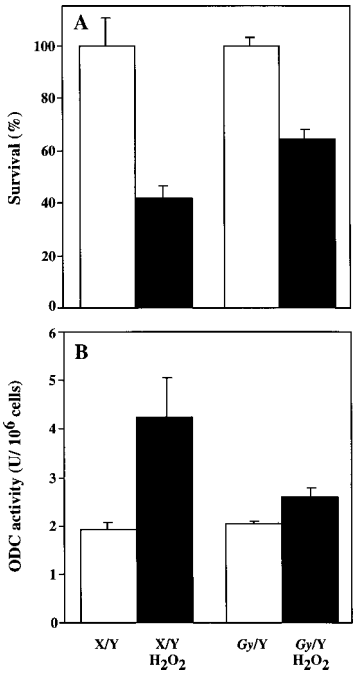


**Figure 6 Effects of oxidative stress on cell survival and on ODC activity**

*Gy*/Y and normal X/Y cells were cultured in the absence or presence of 250 $\mu$M $H_2O_2$ for 24 h. Cells were counted in a haemocytometer (**A**) and ODC activity was determined (**B**). Results are means $\pm$ S.D. ($n = 3$).

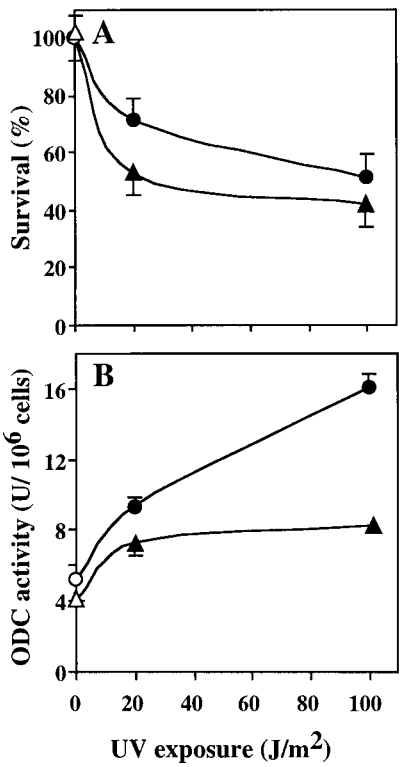


**Figure 7 Effects of UV-C irradiation on cell survival and on ODC activity**

*Gy*/Y cells (△,▲) and their normal X/Y counterparts (○,●) were irradiated with the indicated doses of UV-C light (254 nm). Untreated (△,○) and UV-irradiated cells were cultured for an additional 24 h; they were then counted in a haemocytometer (**A**) and ODC activity was determined (**B**). Results are means $\pm$ S.D. ($n = 3$).

### Effects of oxidative stress on *Gy*/Y cells in comparison with their normal counterpart

Spermine has been recently shown to be a direct free-radical scavenger *in vitro* [8]. In an attempt to verify this hypothesis in cell culture, we treated *Gy*/Y and X/Y cells with $H_2O_2$. When $H_2O_2$ reacts with DNA-associated transition metals the very reactive hydroxyl radicals are generated. These, rather than $H_2O_2$ itself, are believed to be responsible for oxidative toxicity *in vivo*. Contrary to our expectations, *Gy*/Y cells, although lacking spermine, proved to be less sensitive to oxidative stress than were X/Y cells (Figure 6A). When exposed to a $H_2O_2$ concentration of 250 $\mu$M for 24 h, 60 % of the X/Y cells died but only 35 % of the *Gy*/Y cells did so. Accordingly, when measuring the ODC activity (as a marker of stress [12]) in the cells that survived, we found that the X/Y cells exhibited a 110 % increase in ODC activity, whereas *Gy*/Y cells exhibited only a 20 % increase (Figure 6B). When attempting to increase the cellular spermidine content of the X/Y cells (to resemble the situation in the *Gy*/Y cells) by adding spermidine to the culture medium, we observed massive cell death. This was probably due to the presence of serum amine oxidases in the culture medium, which can generate highly toxic polyamine metabolites. Even a 1 mM concentration of aminoguanidine, a serum amine oxidase inhibitor, did not prevent cell death from occurring, possibly because *Gy*/Y and X/Y cells require a very high concentration (20 %) of fetal calf serum for growth.

### Effects of UV-C irradiation on *Gy*/Y cells in comparison with their normal counterpart

UV radiation is known to cause lesions in DNA strands. It is conceivable that spermine, being the largest of the polyamines present in mammalian cells and the one most strongly bound to DNA, has a role in protecting cells against DNA damage. Indeed, when we exposed *Gy*/Y cells and X/Y cells to 254 nm UV-C radiation, we found that the spermine-deficient *Gy*/Y cells were more sensitive than the X/Y cells (Figure 7A). At a UV-C dosage of 20 J/m², 47 % of the *Gy*/Y cells were killed, compared with 29 % of the X/Y cells. The difference between the cell lines was not quite as apparent when the cells were exposed to the higher UV-C dosage of 100 J/m². This finding indicates that additional effects appear at higher dosages of UV-C irradiation. As with oxidative stress, ODC activity was induced to a greater extent in the X/Y cells than in *Gy*/Y cells (Figure 7B). X/Y cells exhibited a more than 200 % increase in ODC activity when exposed to 100 J/m², whereas *Gy*/Y cells exhibited only a 100 % increase. When attempting to generate a spermine pool in the *Gy*/Y cells (to compensate for the deficiency of the spermine synthase gene) by adding spermine to the culture medium, we encountered the same problem with serum amine oxidases as described above for spermidine addition, i.e. massive cell death. Attempts to transfect a mouse spermine synthase cDNA into the *Gy*/Y cells have so far failed, mainly because these cells proved difficult to transfect.

## DISCUSSION

The *Gy* mutation was described as another cause of X-linked hypophosphataemia in the laboratory mouse [22]. Previously the *Hyp* mouse had served as the sole animal model for this disease, showing hypophosphataemia as a result of impaired renal tubular reabsorption of phosphate, and vitamin D non-responsive

rickets/osteomalacia [38]. When the phenotype of *Gy* mice was compared with that of *Hyp* mice, many additional defects became apparent. Although the *Hyp* and *Gy* mutations both affect the *Phex* gene, which encodes a neutral endopeptidase with significant homology to members of the membrane-bound metalloendopeptidase family, the *Gy* deletion extends far beyond the *Phex* gene and includes the upstream neighbour, the spermine synthase gene [25–27]. In fact, the deletion encompasses the entire spermine synthase gene and approx. 50 kb upstream of the first exon [26]. The resulting spermine deficiency can probably explain most, if not all, of the additional defects seen in *Gy*/Y mice. That *Gy* males are smaller than *Hyp* males and normal littermates at all times from birth onwards [22] is in agreement with the contention that spermine is essential for normal cell growth and division [39]. The typical circling behaviour of *Gy*/Y mice, which results from inner-ear abnormalities, as well as their other neurological disorders [22], might relate to the finding that spermine is a modulator of ion channels in the brain [40]. Another parallel is that *Gy*/Y males seem to be deaf, as indicated by an absent Preyer reflex [22], and most patients treated with DFMO experience reversible deafness [41]. Electrophysiological and histological studies on guinea pigs suggest that the anatomical site of DFMO-induced hearing loss is the organ of Corti [42], which is consistent with the fact that *Gy*/Y mice have abnormalities in the organ of Corti. The reason for the observed sterility of *Gy*/Y males [22] remains unknown. As expected in a spermine-deficient animal with decreased body weight, there is also a decrease in testis weight and sperm number. Nevertheless, *Gy*/Y males are capable of producing vaginal plugs. Interestingly, the inhibition of polyamine synthesis causes an arrest of mouse embryogenesis and subsequent resorption of the fetuses [43]. Other characteristics of the *Gy* male include: decreased viability from birth, sudden death in adults, head shaking, lack of postural reflexes, extreme hyperactivity and abnormal head shape. Future studies will establish whether these characteristics also are due to spermine deficiency. The present study addressed the question of whether skin fibroblasts derived from *Gy*/Y mice exhibit increased sensitivity to oxidative stress and UV irradiation because of their lack of spermine. Studies *in vitro* have shown that spermine can protect DNA against hydrodynamic shear [44], heat denaturation [45], UV-C irradiation [46], singlet oxygen [47,48] and other free radicals [8]. We were therefore surprised to find that *Gy*/Y cells, which have no detectable spermine, were more resistant to $H_2O_2$ exposure than their normal counterparts. This effect was not attributable to differences in the levels of GSH between the two cell lines, which were low in both cell lines [$20.0 \pm 6.8$ pmol of GSH/$\mu$g of protein ($n = 3$) in the normal cells and $21.3 \pm 2.8$ pmol of GSH/$\mu$g of protein ($n = 3$) in the *Gy*/Y cells; values are means $\pm$ S.E.M.]. Therefore the protective effect seen in *Gy*/Y cells is probably due to the compensatory, 4-fold, increase in spermidine content and the decreased ability to induce SSAT, in other words polyamine catabolism. Indeed, it was shown recently that not only spermine but also spermidine possesses antioxidant properties *in vitro*, if present at a higher concentration [49]. Our finding, that *Gy*/Y cells accumulate spermidine and are less sensitive to oxidative stress, strongly indicates that spermidine is a potent free-radical scavenger in cell culture. Free radicals are also produced in cells after UV-C irradiation [50]. Given the observed protective effect of the compensatory spermidine accumulation against oxidative stress, we were surprised to find that the *Gy*/Y cells were more sensitive to UV-C irradiation than their normal counterparts. It therefore seems that a lack of spermine increases susceptibility to the genotoxic action of UV-C radiation. It is particularly interesting to note that the hemizygous *Gy* mouse was identified as one of the radiosensitive mutants among 26 genetically well-defined mouse mutations [51]. It is conceivable that spermine deficiency is the reason for this radiosensitivity of *Gy*/Y mice. UV-C-induced cytotoxicity is somewhat pleiotropic, with pathways converging into the activation of c-*jun* N-terminal kinases, the activation of caspases and apoptotic cell death [52]. In addition to the production of free radicals, the initial insults caused by UV-C irradiation include photochemical thymidine dimer production and ribotoxic stress response [53]. Taken together, our results suggest that free-radical production resulting from UV-C exposure is of little importance in the *Gy*/Y cells because of their resistance to oxidative stress. These results are consistent with the notion that oxidative stressors and UV radiation trigger the activation of different signal transduction pathways [54]. Cellular defence lines against DNA-damaging agents such as UV radiation or oxidative-stress-derived free radicals include (1) catalase, superoxide dismutase and peroxidase, (2) antioxidant molecules and (3) the DNA repair machinery. An increasing number of molecules have been shown to exhibit antioxidant properties, such as glutathione [55] and metallothioneins [56]. Here we provide evidence for a generally important role *in vivo* for the polyamines in the protection against oxidative stress and a more specific role *in vivo* for spermine in protection against UV-C light.

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