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Elevated DNA Excision Repair Capacity in the Extraembryonic Mesoderm of the Midgestation Mouse Embryo

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Abstract

In order to determine whether there is differential cell-type-specific DNA repair we measured the nucleotide excision repair capacity of the four distinct cell lineages that comprise the extraembryonic yolk sac using the unscheduled DNA synthesis assay. Yolk sacs from mouse embryos at 11.5–12.5 days gestation were microdissected to yield purified trophoblast, parietal endoderm, mesoderm, and visceral endoderm, as well as fetal skin fibroblasts which were then grown as primary explants. At this midgestational stage of development, the yolk sac provides essential functions for the sustenance of the embryo while the complex process of organogenesis is proceeding in the liver, kidney, and gut. Trophoblast giant cells, parietal endoderm, and visceral endoderm all demonstrated low levels of unscheduled DNA synthesis consistent with levels measured in adult mouse skin fibroblasts. As has previously been documented, embryonic mouse skin fibroblasts were reproducibly 2- to 3-fold higher than adult mouse skin fibroblasts in levels of DNA excision repair. The extraembryonic mesoderm, however, displayed a statistically significant level of unscheduled DNA synthesis 10-fold higher than adult mouse skin fibroblasts or the other lineages of the midgestation yolk sac. Further, the S-indexes of these lineages were also determined to assess the possible relevance of differential repair to the proliferative status of the cells. These data demonstrate that DNA excision repair capacity is lineage-specific during embryogenesis in the mouse. These studies may begin to provide a context for understanding the perplexing developmental aspects such as the characteristic congenital abnormalities associated with the human heritable DNA repair deficiency diseases.

INTRODUCTION

Living organisms are subject to an ongoing process of DNA damage and repair, with damage caused by exposure to a wide range of exogenous chemical and physical agents, as well as endogenous genotoxic processes. Nucleotide excision repair (NER) is one of the

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primary metabolic pathways of DNA repair and is the process responsible for the removal of pyrimidine dimers and 6-4 photoproducts generated by exposure of DNA to ultraviolet light and certain drugs. This complex process is mediated by 20–30 enzymes which perform a series of sequential steps including recognition of the damage lesions, chain incision, excision of a 29- to 30-nucleotide segment around the site of damage, repair replication, and ligation [1–4]. The physiological relevance of this process was made apparent by the discovery that cells from patients with the inherited disease xeroderma pigmentosum exhibit impaired UV-induced repair of DNA, which is associated with UV-induced carcinogenesis [5, 6].

The question of whether there is cell-type-specific DNA repair during embryogenesis has not been directly addressed using purified cell types. Indirect evidence from repair deficiency disorders such as xeroderma pigmentosum, ataxia telangiectasia, and Bloom syndrome which manifest developmental problems such as mental retardation, disproportionate limbs, missing thumbs, and lymphoreticular disorders such as immune insufficiency and early onset cancers suggests that reduced repair may affect some lineages and cell types more than others.

There is evidence from the literature that differential repair occurs in adult cell types. This evidence is usually linked to the proliferative status of these cells. Bowman [7] has classified mammalian cells as belonging to one of four groups: (I) rapidly renewing cells that have a generation time of less than 30 days (colon epithelium cells, bone marrow stem cells); (II) slowly renewing cells that have a generation time of greater than 30 days but less than the mean lifespan of the animal (respiratory tract epithelium and fibroblasts of the dermal connective tissue); (III) cells that have a slow rate of renewal, not necessarily renewing themselves during the lifespan of the animal (smooth muscle cells and the glial cells of the brain); and (IV) cell populations that are terminally differentiated such as neurons, cardiac muscle cells, and skeletal muscle cells. Alexander [8] was the first to speculate that DNA repair is turned off during mammalian development as cells differentiate to the postmitotic state. There is evidence that the DNA repair capacity of muscle cells is lower in postmitotic cells than in proliferating cells from embryonic or newborn rats [9, 10]. However, these studies have used a variety of DNA damaging agents and these agents give different results. For example, myoblasts undergoing differentiation to multinucleated contracting myotubes in culture decline in the capacity to repair 4-nitroquinoline-1-oxide damage, but not X-ray- or UV-induced damage [11, 12].

The question of whether differences in NER exist due to the differentiative potential or status within a given cell type or lineage has never been clearly answered. Studies using chick muscle cells exposed to UV light [9] and rat muscle cells exposed to methylmethane sulfonate [13] or 4-NQO [11] have suggested that DNA repair efficiency decreases with terminal differentiation. However, Kaufman *et al.* [14], studying unscheduled DNA synthesis (UDS) after UV irradiation in a rat L8 muscle cell subclone, have disputed these findings, observing instead constant levels of repair throughout differentiation except for a transient period of increased repair immediately following the cessation of replicative DNA synthesis. A study by Ho and Hanawalt [15] attempted to address this question using two different methods of measuring DNA repair in terminally differentiating L8 rat myoblasts:

(i) repair replication and (ii) removal of T4 endonuclease V-sensitive sites (ESS). The results of this study concur with those of Kaufman *et al.* [14] in that there were no significant differences detected in ESS removal between exponentially growing myoblasts and that in differentiating myotubes. Further, very little overall repair occurred during both periods.

The ultimate model of differentiation for any cell type is the developing embryo. The pattern or role of NER has never been defined in specific lineages during mammalian development, despite the fact that several human DNA repair deficiency syndromes manifest developmental abnormalities [16]. Studies of NER in mixed lineages during development using the functional assay for NER, UDS, have shown that in contrast to established mouse cell lines, primary murine embryonic fibroblasts excise dimers at a greater rate [17,18] and perform higher levels of repair synthesis [19]. Early passages of mixed embryonic fibroblasts have been shown to manifest higher levels of excision repair than later passages [17]. Peleg *et al.* [18] have since shown that the capacity for excision repair of UV radiation damage in passaged embryonic fibroblasts from mouse is dependent upon the gestational stage and the duration of *in vitro* growth. This study was performed on repeatedly passaged mixed primary embryo fibroblasts at 13–15 or 17–19 days gestation (dg).

In contrast to these studies, our study was designed to determine the overall excision repair capacity of purified primary lineages within a specific midgestational stage (11.5–12.5 dg) using the UDS functional assay. One of the advantages of utilizing a functional assay for NER is that this assay can effectively survey the functioning of all the gene products (approximately 20–30) that comprise damage recognition and repair for this pathway. We have evaluated unpassaged embryonic lineages as well as extraembryonic lineages of the visceral or parietal yolk sacs from midgestational embryos and compared them with normal human and mouse skin fibroblasts.

DNA repair capacity during normal embryogenesis is clearly important since many of the phenotypes of heritable DNA repair-deficient diseases include evidence of significant developmental disruption such as the neurological abnormalities manifested in xeroderma pigmentosum, ataxia telangiectasia, and Cockayne syndrome and the failure of the immune system and erythroid lineages of Fanconi anemia and other repair syndromes [16]. Such studies will also be valuable for anticipating and interpreting the phenotypes of genetically engineered mice that are specifically mutagenized in genes which have been shown to function at some level in the complex process of NER [20].

MATERIALS AND METHODS

Mice

Pregnant ICR female mice, stock DUB (originally obtained from Dominion Laboratories, Inc., Dublin, VA) or CD-1 (originally obtained from Charles River Laboratories, Wilmington, MA), were used to generate embryos of 11.5 and 12.5 dg. Both stocks were maintained as closed colonies in our animal facility. Animals were maintained on a cycle of 14 h light, 10 h dark (light period, 6:00 A.M. to 8:00 P.M.). All embryos were obtained after natural mating. Noon of the day the vaginal plug was detected was designated 0.5 dg.

Media

Dissections were performed in FM-II embryo culture medium [21], a modification of embryonic stem cell media [22]. UDS assays were performed using MEM supplemented with Earles' balanced salts solution, 10% fetal calf serum, and $10 \mu\text{Ci ml}^{-1}$ [*methyl*- ^3H]thymidine (80 Ci mmol^{-1} ; Dupont NEN) as labeling medium. Chasing medium was identical except that it contained 10^{-3} M nonradioactive thymidine rather than [*methyl*- ^3H]thymidine.

Dissections of embryonic and extraembryonic tissues

Midgestational 11.5- and 12.5-day embryos within decidua were dissected out of the uteri of sacrificed pregnant females. The decidua were then dissected off the extraembryonic structures, leaving the yolk sac essentially intact. Yolk sacs were then dissected from the embryo, and the amnion was subsequently removed and discarded, rinsed in sterile PBS, and finally placed in 0.5% trypsin (Calbiochem, San Diego, CA), 2.5% pancreatin (Sigma Chemical Co., St. Louis, MO) in Ca^{2+} - and Mg^{2+} -free PBS (CMF-PBS) [23, 24] for 1–5 h at 4°C . This enzymatic treatment served to loosen the connections between the cell layers. The various layers could then be separated from one another using fine watchmakers forceps and a dissecting microscope after the tissues were rinsed in serum-containing FMII embryo culture medium. Parietal endoderm combined with trophoblast cells was removed from the visceral endoderm and the inside of the decidua. Mesoderm was separated from visceral endoderm.

Primary explant cultures of dissected lineages

Newborn human fibroblasts were derived from normal human foreskins and grown continuously in culture. These cultures show constant levels of DNA repair until senescence after approximately 20 passages [25]. Adult mouse fibroblasts were derived from the ears of ICR adult female mice by mincing the ear with sterile scissors and plating the clumps into prewarmed medium on fibronectin-coated two-chamber slides (Lab Tek, Miles Laboratories). Embryonic fibroblasts were derived by dissecting the fetus away from the extraembryonic structures such as the amnion, yolk sac, and ectoplacental cone. The embryos were treated as described by Robertson [22] and then trypsinized for several hours and triturated by pipetting repeatedly until they were a single-cell suspension. These cells were used within the first three passages in culture.

Visceral yolk sac mesoderm was dissected from visceral extraembryonic endoderm after incubation in trypsin–pancreatin (0.5% trypsin and 2.5% pancreatin). Sheets of mesodermal fibroblasts from the yolk sac were stripped free of visceral endoderm cells and torn into small clumps. These clumps were then placed into prewarmed medium in two-chamber slides that had been previously coated with fibronectin (Boehringer Mannheim).

It was not possible to grow monolayer cultures of visceral endoderm as these cells would not flatten and attach to coated glass slides. Therefore a variation of Tarkowski's method [26, 27] was used to fix the irradiated nuclei of the visceral endoderm layer to slides for autoradiography. This protocol consisted of exposing the dissected visceral endoderm sheets to hypotonic solution for 5–10 min (0.075 KCl), after which they were fixed with

methanol:acetic acid (3:1) for 10 min. This was then replaced with 60% acetic acid in water. Nuclei were then deposited on a flat slide prewarmed at 37 to 40°C. In order to evaluate whether this method of performing the UDS assay, i.e., isolation and fixation of nuclei after UV irradiation, would give rise to results consistent with the previous method, extraembryonic mesoderm was prepared in a similar manner.

Unscheduled DNA synthesis and autoradiography

DNA damage occurs constantly in mammalian cells due to irradiation, chemical exposure, and the products of normal metabolism. Damage remediation also occurs ubiquitously to prevent fixation of DNA damage into mutation during the process of replication. Nucleotide excision repair, a well-characterized pathway of DNA repair (UV repair of 6-4 photoproducts and pyrimidine dimers) was measured using autoradiography of UDS [28]. Primary cultures of explanted yolk sac mesoderm, fetal skin, parietal endoderm (combined with trophoblast giant cells), and human and mouse adult skin fibroblasts were established on fibronectin-coated glass slides and allowed to spread, forming a monolayer. After a total of 36 h in culture without passaging of the cells, these cultures were irradiated with 254 nm UV light at a mean fluence of $1.3 \text{ J/m}^{-2} \text{ s}^{-1}$ in the absence of culture medium for a total dose of 13 J/m^{-2} . This dose of UV light was administered using a specially designed exposure machine [29] constructed such that the germicidal bulbs are placed at a distance of 3 ft away from the turntable where the cultures are placed for irradiation. Exposure to the UV light is performed via a precisely timed electronic shutter opening and closing, and the total time of exposure is 10 s. In this brief exposure time at a distance of 3 ft, there is no temperature increase near the living cells. This machine was designed to minimize the amount of heat and produced a precisely delivered highly reproducible dose of UV-C light.

One chamber of each double-chambered slide was left unirradiated as a control. All culture chambers were incubated in medium supplemented with 10 mCi ml^{-1} [*methyl*- ^3H]thymidine (80 Ci mmol^{-1} ; Dupont NEN) for 2 h at 37°C under 5% CO_2 . Labeling medium was replaced with chasing medium containing 10^{-3} M thymidine and incubated for 2 h to clear label from the intracellular nucleotide pools.

Visceral endoderm cells are asymmetric with the nucleus located closer to the mesoderm side (i.e., basolateral side) of the visceral yolk sac (VYS). Therefore, sheets dissected from the mesoderm layer were placed with the basal side up on dry chamber slides and subsequently irradiated with 26 J/m^2 UV. This higher dose was necessary to deliver the UV through the complex secondary structure of the intact visceral endoderm. As a control, extraembryonic mesoderm and human fibroblast monolayers were similarly irradiated and processed through the UDS assay. The nuclei from these cell types were then isolated and fixed as described previously [26, 27].

After incubation in the postlabeling medium of the UDS assay, all cells were fixed in 33% acetic acid in ethanol followed by 70% ethanol and finally in 4% perchloric acid overnight. Fixed nuclei prepared by the modified Tarkowski method were placed in 4% perchloric acid for 1–2 h or overnight. All slides were dried and subsequently dipped in photographic emulsion (Kodak type NBT) and exposed for 10 to 14 days in complete darkness at 4°C. Slides were developed in D19 and washed once with 2% acetic acid and water.

Grain counting and S-index quantitation

Nuclei were stained with Giemsa and examined at 800–1000× magnification for grains over the nuclei of non-S-phase cells as a measure of UDS indicative of NER. For each cell type assayed, grains were counted over at least 75 nuclei in monolayer regions of the slide. Background grain counts were evaluated over an equivalently sized region adjacent to the nuclei or cells and were subtracted from all measurements. The mean counts from the unirradiated chamber of each two-chamber slide were subtracted from the grain measurements of the irradiated chamber. Final grain counts are expressed as a mean of the corrected irradiated cultures or displayed as a histogram of corrected counts with the calculated standard error. The standard error was calculated using the standard formula for combining two standard errors (i.e., the standard error derived from the mean irradiated counts and the standard error of the mean unirradiated counts).

Nuclei undergoing S-phase, in our hands, appear black (>100 grains per nucleus) relative to nuclei that were not undergoing S-phase at the time of labeling. The percentage of cells in S-phase was determined in each microscopic field by counting the nuclei in S-phase divided by the total number of nuclei.

Statistical analysis of UDS data

Statistical analyses of the data derived from the UDS analysis (ANOVA and Student's *t* test) were performed using Microsoft Excel. The two-tailed *t* test was performed assuming unequal variance.

RESULTS

The mouse yolk sac, unlike that of the human, encircles the entire embryo, and is exterior to the amniotic sac. The yolk sac consists of an outer parietal yolk sac (PYS, Fig. 1A) and an inner visceral yolk sac layer (VYS, Fig. 1A), both of which can be further separated into two distinct cell types: the parietal layer into trophoblast and parietal endoderm, and the visceral layer into visceral endoderm and mesoderm (Fig. 1B). Specific lineages were dissected from the 11.5- to 12.5-dg mouse yolk sac via a trypsin–pancreatin digestion [23, 24, 30] and explanted into culture [31]. Cells from three lineages, mesoderm, parietal endoderm, and trophoblast, were able to attach and spread onto a fibronectin-coated chamber slide and ultimately form a discontinuous monolayer (Fig. 2). Control explants of fetal and adult skin fibroblasts formed continuous monolayers in this explant culture system (Fig. 2).

One of the benefits of performing the classical UDS assay on these embryonic and extraembryonic cell types is that in postlabeling one can verify the identities of these cells based on their morphological characteristics and differences. In many cases, previous studies using different batch methods for evaluating DNA repair may have combined different cell types which were capable of growing in culture. In the case of the trophoblast giant cells, the cytoplasm and the nucleus of these cells are markedly larger than those of other lineages. This is due to the nature of trophoblast cell DNA replication which consists of DNA synthesis without cell division, a process known as endoreduplication.

UDS was measured in these cell types in a manner similar to that used for normal human foreskin cells [28] (Figs. 3 and 4). Cell monolayers were exposed to UV_{254nm} irradiation at a mean fluence of $1.3 \text{ J/m}^{-2} \text{ s}^{-1}$ for a total dose of 13 J/m^{-2} and then allowed to incorporate [³H]thymidine during repair synthesis. After the fixed slides were dipped in photographic emulsion, the resultant silver grains were quantitated visually and are proportional to the level of NER. S-phase nuclei of irradiated and unirradiated cells of all lineages tested were heavily labeled and non-S-phase cells were differentially labeled, depending on the amount of NER which had occurred. Human foreskin fibroblast (ff) cells were included in all experiments to serve as a standard for comparison for all of the mouse cell lineages. Adult mouse skin was also included in these studies to serve as a reference point for a level of UDS that has been previously documented in murine skin fibroblasts [17, 18, 32, 33].

Several levels of background correction were performed on the raw data. Initially, corrections for background were made by subtracting the number of grains present in an equivalent area adjacent to each nucleus, and subsequent calculation of a mean grain number. Mean grain numbers from the control (nonirradiated chamber of the slide) as well as the mean from the irradiated chamber of the slide were calculated. The mean from the nonirradiated side of the slide was then subtracted from a similarly calculated mean grain count on the irradiated side of the same slide.

In the case of visceral endoderm, the cells were not amenable to attachment, even in the presence of three different commercially available extracellular matrix molecules. Since it is possible to culture the yolk sac without attachment to a substrate [34], the UDS assay was performed on tissue that was spread intact and unattached upon a glass slide. Since the nucleus of a visceral endoderm cell is polar (see Fig. 1B), the sheet of visceral endoderm cells was oriented nucleus side up, such that the nuclei received the maximum UV dose possible. As a control, extraembryonic mesoderm and human foreskin fibroblast monolayers were similarly irradiated (as detached cell layers) and processed through the UDS assay. This modified Tarkowski technique [26] was first used by Pedersen and Cleaver [27] to study NER in mammalian pre- and postimplantation stage embryos.

Histograms of the distribution of grain counts in each lineage, after both levels of correction for background, are given in Figs. 5 and 6. The normal human foreskin cells had means in the range 25 to 30 grains/nucleus (Fig. 5A) compared to the adult mouse skin which had a mean of 1 grain/nucleus (3.5% FF) (Fig. 5B). The 25-fold difference between human and mouse primary skin cells has been documented by others and may reflect a fundamental difference in the extent to which these respective mammalian species deal with UV damage, presumably at the level of the noncoding genomic repair of pyrimidine dimers and 6-4 photoproducts [35, 36].

The range of UDS observed in the explanted murine mesoderm cells of the visceral yolk sac was strikingly higher than any other lineage tested in this study (Fig. 6B). This lineage had a mean of 9 grains/nucleus (37% of foreskin fibroblasts), representing a level of UDS that was 3-fold higher than the next highest level of UDS documented [17, 18] namely that of the primary fetal skin fibroblasts (12.5% FF) (Fig. 6A). Normal human foreskin fibroblast cells were used as a positive standard of comparison in every experiment to allow

interexperimental comparisons. All means were converted to a percentage of this standard and a summary of the mouse cell types tested in five independent experiments is shown in Fig. 7. Other extraembryonic lineages of the yolk sac, trophoblast, parietal endoderm, and visceral endoderm were 10-fold lower than the mesoderm in NER as measured by this assay. Despite the size of the nuclei of the trophoblast giant cells, grain counts were still low after background normalization for this lineage.

ANOVA analysis of all seven cell types (foreskin fibroblasts, mesoderm, visceral endoderm, fetal fibroblasts, adult mouse skin, trophoblast, and parietal endoderm) indicated overall statistical differences between these lineages ($P < 0.0001$). Two-tailed pairwise Student's t tests indicated that there were statistically significant differences ($P < 0.0001$) in all pairwise permutations between the lineages except between visceral endoderm and mouse skin ($P = 0.08$), visceral endoderm and trophoblast ($P = 0.27$), and parietal endoderm and trophoblast ($P = 0.33$).

As an indicator of the cell cycle differences between the lineages analyzed by UDS in this study, an S-index, or the relative number of cells undergoing S-phase relative to the total number of cells or nuclei, was determined for each of the four murine cell types and human foreskin fibroblasts (Table 1). Although murine fetal skin and parietal endoderm had high S-indices, they manifested a low capacity for NER. All of the differences between S-phase indices from the different cell types were significant as determined by pairwise Student's t tests ($P < 0.01$) except that of fetal skin vs human foreskin fibroblasts.

DISCUSSION

DNA damage of all kinds occurs constantly in all cell types due to environmental agents, irradiation, and the normal products of metabolism. Repair processes are ubiquitous and constitutive in their activity in mammalian cells for this reason. We used UV light simply as a way to administer well-documented and defined types of DNA damage, 6-4 photoproducts, and pyrimidine dimers. The kinetics of repair remediation for these types of damages are well documented and measurable without ambiguity. The use of UV mimetic drugs for this purpose such as AAF (*N*-acetoxy-*N*2-acetylaminofluorene) impact on multiple pathways of DNA repair and therefore would not have given us a result specific to the nucleotide excision repair pathway. Our study does not purport that UV light is present *in utero*, simply that the repair remediation for two specific types of DNA damage lesions (6-4 photoproducts and pyrimidine dimers) operate at different levels in different lineages during development. These types of damage can be produced by chemicals (that could be present *in utero*) as well as UV light.

In the context of transgenic mice that are being generated, particularly those that are null mutants for a specific DNA excision repair gene, the determination of differential DNA repair during mouse development is important. In particular, such determination will help in predicting and interpreting the deleterious effects in specific lineages and potential embryonic lethality of specific mutations.

In the case of the XP-A^{-/-} transgenic mouse, it has been shown by De Vries *et al.* [37] that 50% of the embryos from these mice die of severe anemia at midgestation. Histopathological examination of these embryos has shown that the liver is abnormal in its hematopoietic function. In the context of our study, this hematopoietic abnormality may be speculatively explained. The extraembryonic mesoderm, which we have shown has a high level of NER, gives rise to the hematopoietic stem cells at 7.5–8 dg which subsequently migrate from the mesoderm layer to colonize the fetal liver which at 10 dg appears as a distinct hematopoietic organ. Although the blood islands of the mesoderm are only one component of the mesoderm, both blood islands and nonblood island cells arise from the same progenitor cells as shown by lineage analysis [38]. The switch from fetal to adult hemoglobin production in the mouse has been shown to be due to a switch from the yolk sac to fetal liver erythropoiesis [39]. The stem cells of the liver then expand and differentiate to populate the thymus and bone marrow [40].

DNA repair capacity during development [27] has been elucidated in a study that determined that the excision repair capacity of pre- and postimplantation stage murine embryos is low in the nuclei of the morulae, inner cell mass, and trophoblast cells. In addition Peleg *et al.* [18] have shown that later gestation (13–15 dg) embryonic fibroblasts have a greater capacity to perform excision repair than adult mouse skin. These results raise the issue of stage-specificity or differentiative status as a factor in excision repair within mammalian cells.

Since there is evidence that increasing passage *in vitro* may compromise the integrity of DNA repair systems in cells derived from organs [18, 25], our assays were performed on primary unpassaged cultures derived directly from embryos and processed within 36 h. Furthermore, we allowed the cells to retain some of their previous extracellular associations by introducing them into culture as purified tissue aggregates that could grow out upon artificial extracellular matrix substrates, in effect, more like organ culture than cell culture.

We have found that the extraembryonic mesoderm represented the highest level of UDS yet found in any cultured rodent cell line or primary murine cells, whereas fetal skin had UDS that was about 10% of human levels, a level consistent with other studies [17, 18]. The adult mouse skin cells represented the lowest level of UDS in this study (less than 1% of human UDS), which is consistent with other studies of mouse skin [17, 18]. These data suggest that lineage- or cell-type-specific NER exists in mammals, a finding that is important to consider in the context of mutator gene theories of cancer proneness. UDS studies on lymphocytes have also suggested that cell-type-specific repair exists, and that age is a factor [41].

We hypothesize that subtle defects in DNA repair may contribute to the occurrence of a broad range of hematopoietic abnormalities manifested in the neonatal and postnatal period. Profound developmental abnormalities involving anemia, immune insufficiency, and early onset cancers of the lymphopoietic system are characteristic of children with hereditary defects in DNA repair, such as the rare diseases ataxia telangiectasia, Bloom syndrome, and Fanconi anemia, but including more common disorders such as Down syndrome.

To evaluate whether the proliferative potential of cells is correlated with the amount of repair capacity in the yolk sac, we also measured the number of S-phase cells in each of the cell lineages tested. The cells having the highest number of S-phase cells were the human foreskin fibroblasts, although interestingly, these cells had a very similar S-index to that of the murine fetal skin fibroblasts, a cell type having a much lower NER capacity and a similar morphology. Within the context of the murine embryo at midgestation, it seems that NER capacity does not necessarily correlate with proliferative potential. This fact is consistent with other studies that found that NER does not correspond to the differentiative status [15].

These observations of developmental variations in DNA repair and differences in overall repair between mouse and human cells raise questions about the molecular basis for the differences and their functional significance. Mutations in human DNA repair genes that have been identified reduce DNA repair in human cells to a low level similar to that in mouse. Specific missense mutations in the XPAC gene [42] and inactivating mutations in the XPCC gene [43] produce human cells with reduced repair of dimers in the untranscribed region of the genome. Variations in the expression of these genes in different species could be involved in the observed differences in overall repair between species and at different developmental stages. It is possible that the greater capacity of the mesoderm cells at midgestation could reflect the fundamental importance of this tissue as a protective and detoxification organ, as well as its pivotal role in the genesis of the blood and immune systems, which are specifically affected in humans that are genetically deficient in certain excision repair capacities.

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FIG. 1.

(A) Photomicrograph of an intact mouse embryo at midgestation including the extraembryonic yolk sac (12 $\times$ magnification). The maternal deciduum has been dissected off, leaving the underlying yolk sac intact. (A) shows the area of the yolk sac which was sectioned for B (see the rectangle in A). This area shows that the placenta overlaps the yolk sac in the area just peripheral to the implantation site (opaque area on the left of A). The vertical backbone of the embryo can be clearly seen. The areas of the parietal yolk sac (PYS) and visceral yolk sac (VYS) are indicated. The area in the rectangle over the parietal yolk sac has been sectioned and enlarged in (B) (250 $\times$ magnification). This section shows the multiple layers/lineages of the parietal yolk sac. The cell layer furthest to the right (and closest to the maternal uterine wall) is the trophoblast (T), followed by parietal endoderm (PE), visceral endoderm (VE), and the mesoderm (M) which includes endothelial cells (E) surrounding blood vessels. Arrowheads indicate the nucleus of each type of cell.

FIG. 2.

Monolayer cultures of different cell types after microdissection of the embryonic and extraembryonic tissue (all at 400 $\times$ magnification) showing distinct morphologies and characteristics of each cell type. The four cell types pictured are: (A) fetal fibroblasts (embryonic); (B) mesoderm (extraembryonic); (C) parietal endoderm (extraembryonic); (D) single trophoblast cell (extraembryonic).

FIG. 3.

1000× Magnification of cell monolayers after UDS. S-phase nuclei appear to be black. Non-S-phase nuclei show discrete silver grains which correlate with the level of NER. Dosage of UV_{254nm} was 13 J/m². Silver grains are pictured over the following unirradiated and irradiated cell types: (A) unirradiated human skin fibroblasts (control); (B) UV-irradiated human skin fibroblasts; (C) unirradiated mouse 11.5-day extraembryonic mesoderm (control); (D) UV-irradiated mouse 11.5-day extraembryonic mesoderm.

FIG. 4. 1000× Magnification of fixed nuclei after UDS derived from visceral endoderm cells that would not attach to fibronectin-coated chamber slides. These nuclei were fixed and isolated from the living cells after the UDS assay using a modification of the Tarkowski protocol [27]: (A) unirradiated 11.5-day visceral endoderm; (B) UV-irradiated 11.5-day visceral endoderm. Although the mesoderm layer did attach to fibronectin, we performed the same fixation and isolation on mesoderm cells: (C) unirradiated 11.5-day mesoderm; (D) UV-irradiated 11.5-day mesoderm to verify that the UDS results were the same regardless of how the mesoderm cells were grown in explant culture and to provide a standard of

comparison with the visceral endoderm cells. All cell types are extraembryonic. The dosage of UV_{254nm} was 26 J/m².

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FIG. 5. Histograms of normalized grain counts for adult tissues. A normal unimodal distribution of grains per nucleus vs percentage of nuclei was observed for: (A) human foreskin fibroblasts (mean = 25) and (B) murine adult skin fibroblasts (mean = 1).

FIG. 6. Histograms of normalized grain counts for fetal tissues. A normal unimodal distribution of grains per nucleus vs percentage of nuclei was observed for: (A) murine fetal fibroblasts (mean = 3); (B) murine extraembryonic mesoderm (mean = 9).

FIG. 7.

Summary of the mean UDS levels of a subset of lineages expressed as a percentage of human foreskin fibroblasts. Error bars represent standard error. $N = 100$ nuclei for all lineages shown. The standard error for human fibroblasts was 9%.

TABLE 1

Cell type	Mean S-phase nuclei (<i>n</i> = 100)
Extraembryonic mesoderm	19.3%
Parietal endoderm	30%
Trophoblast	1.8%
Fetal skin	50.9%
Human foreskin fibroblasts	54.5%