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Enucleation of Feeder Cells and Egg Cells with Psoralens

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Abstract

The cell nucleus must be inactivated or destroyed in order to generate feeder layers for cultured cells or to prepare recipient egg cells for nuclear transfer. Existing enucleation techniques are either cumbersome or employ toxic chemicals. Here we report a new method to enucleate cells by treatment with a psoralen and long-wave ultraviolet light. The technique is >90% efficient and causes little cytoplasmic damage to the treated cell. We have used psoralen treatment to enucleate a wide variety of cells, including eggs, sperm, HeLa cells, and fibroblasts. Colonies of human embryonic stem cells (hESCs) and human keratinocyte precursors grown on psoralen-treated feeders are indistinguishable from those grown on gamma-irradiated or mitomycin-C treated cells. Psoralen enucleation provides a rapid, simple, and non-toxic method to generate feeder cells. The technique is also useful for nuclear transfer studies in species with large eggs whose cleavage divisions are not regulated by cell cycle checkpoints.

INTRODUCTION

Stem cells and other fastidious cell types are often cultured with feeder cells that provide an appropriate niche to maintain them in their natural physiological state (Thomson et al., 1998). Feeder cells are typically gamma-irradiated or treated with the radiomimetic compound mitomycin C in order to prevent them from proliferating and overgrowing the culture. These agents introduce double-stranded breaks or cross-links into the nuclear DNA, thereby interfering with replication and activating checkpoint mechanisms that arrest the cell cycle. The existing techniques to prepare feeders have serious limitations. Gamma-irradiation requires an expensive cesium source that is usually available off-site and requires compliance

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with strict safety regulations. Mitomycin C is highly toxic and requires several hours of treatment to be effective.

In a similar fashion, the egg cell nucleus must be removed or destroyed during somatic cell nuclear transfer experiments (Li et al., 2004). Manual enucleation does not damage mammalian eggs, but it is time consuming, requires technical expertise, and cannot be used for species that have opaque eggs (Liu et al., 2000a). A number of alternatives to manual enucleation have been developed (Gurdon, 1960; Tatham et al., 1995) (Fulka and Moor, 1993; Wang et al., 2001; Kawakami et al., 2003; Vajta et al., 2005; Li et al., 2006), but these are damaging to the eggs (Smith, 1993) and embryonic development after nuclear transfer is frequently abnormal.

We describe a new method to generate feeder layers and enucleate eggs by treating cells with psoralens and ultraviolet light. Psoralens are tricyclic polyaromatic furocoumarin derivatives that intercalate between the base pairs of double-stranded DNA molecules (Cimino et al., 1985). Psoralens form covalent adducts with thymidine residues when irradiated with long-wave ultraviolet (UV) light (300–400 nm). This reaction introduces cross-links between the two DNA strands at d(TpA) sites. Unlike mitomycin C, psoralens are non-toxic and are commonly taken internally to treat psoriasis (Stern, 2007). We reasoned that extensive interstrand crosslinking with psoralens would interfere with DNA replication and arrest cell division. Here we refer to this process as enucleation, even though the nucleus is not physically removed and may remain transcriptionally active.

RESULTS

Psoralen Enucleation of Egg Cells

To see if psoralen treatment would prevent nuclear replication, freshly laid *Xenopus* eggs were incubated in 50 μ M 4'-aminomethyl - 4, 5', 8-trimethylpsoralen (AMT) for 5 minutes and manually rotated so that the white spot (indicating the position of the meiosis II spindle) was facing upward (Figure 1A). The eggs were irradiated from above for 5 minutes with a 100 W UV source outfitted with a 300–400 nm filter. After irradiation, the eggs were fertilized and allowed to develop in vitro. Both diploid and haploid *Xenopus* embryos develop to the swimming tadpole stage and can be distinguished by their physical appearance (Gurdon, 1960) (Figure 1B). Diploid embryos are elongated and tapered while haploid embryos are foreshortened, plump, and edematous. Eggs irradiated in the presence of AMT before fertilization gave rise to tadpoles with a typical haploid appearance (Figure 1C), as would be expected if the egg nucleus had not replicated. Karyotype analysis confirmed that embryos derived from AMT+UV-treated eggs had 18 chromosomes while embryos derived from untreated eggs had the normal diploid complement of 36 (Tymowska and Kobel, 1972) (Figure 1B and Table 1). We established primary cell cultures from pools of 20 tadpoles and measured the DNA content of individual cells by flow cytometry. Somatic cells derived from AMT+UV-treated eggs had a DNA content that was exactly half of that of cells derived from untreated eggs (Figure 1D). Eggs that were treated with AMT only or UV light only developed into normal diploid embryos whose cells had the same DNA content as untreated controls.

To show that the AMT+UV-treated egg nucleus does not contribute genetically to embryonic development, we employed a strain of transgenic frogs that express GFP under the control of the ubiquitous CMV promoter (Marsh-Armstrong et al., 1999). When untreated CMV-GFP(+) eggs were fertilized with GFP(−) sperm, 63% of the embryos (111/176) expressed GFP, indicating that the mother was heterozygous for the CMV-GFP allele (Figure 1E, left). In contrast, when the eggs of a CMV-GFP(+) female were treated with AMT and UV light before fertilization, virtually none of the haploid embryos (n=51) expressed GFP (Figure 1E, right). The single exception was a mosaic that expressed GFP only in a patch of cells along the spine (not shown). Karyotype analysis confirmed that the GFP(−) tadpoles from AMT+UV-treated

eggs had only 18 chromosomes ($n=5$). The karyotype results virtually exclude the possibility that the embryos became GFP(−) due to psoralen-induced point mutations at the GFP locus.

Enucleation of *Xenopus* eggs with psoralen and ultraviolet light is robust and reproducible. Among 20 different clutches of eggs from different mothers, the average efficiency of enucleation was $87 \pm 11\%$. The eggs do not appear to be damaged by psoralen + UV treatment, as evidenced by their high rates of fertilization and development into haploid tadpoles (80–100%). 8-methoxypsoralen (MOP) could be substituted for AMT with little change in the efficiency of enucleation (Figure 1C). The ideal conditions for enucleation of *Xenopus* eggs were irradiation for 2–5 minutes in the presence of 25–50 μM AMT (Figure 2D and 2E). Eggs irradiated for shorter periods of time or with lower concentrations of AMT produced abnormal embryos that either died during early development or gave rise to abnormally shaped tadpoles that resembled neither diploids nor haploids (not shown). Karyotype analysis showed that most surviving embryos had 32–34 chromosomes or were close to triploid (Table 1 and not shown). *Xenopus* eggs can also be enucleated by irradiation with short-wave UV light (Gurdon, 1960), but we found that some clutches of eggs treated in this way fertilized poorly compared to psoralen-treated eggs (not shown).

To show that psoralen-enucleated eggs were suitable recipients for nuclear transplantation, eggs from a CMV-GFP female were enucleated with MOP and ultraviolet light and transplanted with blastula nuclei from control embryos. From 216 primary transfers, we obtained 21 embryos, 10 of which had a normal morphology at stage 40. None of the 21 embryos exhibited GFP fluorescence, indicating that the recipient egg's nucleus had been destroyed by the enucleation procedure (Figure 1F, left). We also performed the converse experiment, in which control eggs were enucleated with MOP and ultraviolet light and transplanted with cells from CMV-GFP blastulae. Of these transplant embryos, 35 of 36 (97%) were GFP(+), indicating that development was directed by the transplanted nucleus (Figure 1F, right). Karyotype analysis revealed that 8 of 9 of these GFP(+) transplant embryos had the normal complement of 36 ± 1 chromosomes, while one had 47 ± 2 chromosomes (Table 1). Thus, the frequency of complete enucleation appears to be about 90%, in agreement with estimates based on haploid analysis.

Psoralen Enucleation of Sperm Cells

To see if psoralens could be used to enucleate other types of cells, sperm from a pigmented *Xenopus* male were treated with AMT and UV light then used to fertilize eggs from an albino female (Figure 2A). Virtually all of the tadpoles that developed had a typical haploid appearance and all were albino, indicating that the sperm nucleus did not contribute to the embryonic genome (Figure 2, B and C). Karyotype analysis confirmed that the haploid-appearing embryos had 18 chromosomes (Table 1). Untreated sperm and sperm exposed to long-wave UV light without psoralen gave rise to normal diploid pigmented embryos (Figure 2B, left). The sperm nucleus is much more sensitive to psoralen and UV light than the egg nucleus. The efficiency of enucleation was higher ($95 \pm 3\%$), and a psoralen concentration as low as 1 μM or an exposure to UV light as short as 15 seconds was sufficient to completely destroy the nucleus (Figure 2, E and F). This increased sensitivity is probably because the sperm nucleus is less shielded by cytoplasm. Indeed, sperm that were treated with AMT but not exposed to UV light sometimes gave rise to abnormal embryos unless fertilization was performed in a darkroom under a safelight (not shown). Apparently, ambient room light contains enough long-wave UV radiation to cause some cross-linking. Treatment of sperm with AMT and UV light had no effect on the efficiency of fertilization (not shown). When both sperm and egg were treated with AMT + UV light, fertilization was normal and the eggs underwent several irregular cleavage divisions before dying during the blastula stage (Figure S1).

We found that psoralens could also be used to enucleate mouse eggs and sperm. Mouse eggs were much more sensitive to psoralens and ultraviolet light than *Xenopus* eggs, probably because they are smaller and more translucent. Both the concentration of psoralen and the dose of UV light had to be reduced from the conditions used for frog eggs; otherwise either treatment by itself would cause adverse effects on embryonic development in the absence of the other treatment (not shown). Eggs from super-ovulated mice were irradiated for 30 seconds with a handheld 4W UV lamp in the presence of 4–6 μ M AMT, then parthenogenetically activated with strontium chloride and cultured in vitro for four days. AMT + UV-treated eggs arrested development at the 1–2 cell stage, while untreated eggs or eggs treated with UV light only or AMT only formed complete blastocysts (Figure 3A). Mouse sperm cells treated with AMT + UV light successfully fertilized mouse eggs in vitro, indicated by the presence of two pronuclei (Figure 3B). The fertilized zygotes, however, rarely cleaved even once, while 20–40% of eggs fertilized with untreated sperm developed to the blastocyst stage. These results indicate that, unlike *Xenopus* eggs, mouse eggs fertilized with AMT+UV-treated sperm arrest development at the first cell cycle. We suspect that the arrest is due to activation of cell cycle checkpoints by the psoralen-damaged DNA (see below). In *Xenopus* embryos checkpoints do not become operational until the 12th cell division (Gerhart et al., 1984), by which time the psoralen-damaged DNA would have been segregated to a small group of cells. In mouse embryos, however, checkpoints are active during the early cleavage divisions (Liu et al., 2000b; Takai et al., 2000).

Production of Feeder Cells with Psoralens

To see if psoralen-treated cells could be used as feeders, we first examined the effects of psoralen + UV treatment on the growth of HeLa cells. Cells were treated with various concentrations of MOP, irradiated with long wave UV light for 1 minute, and then cultured for three days. We found that a dose of 5 μ M MOP would effectively arrest HeLa cell growth while maintaining cell viability at 80–100% (Figure 3C). Flow cytometry of the MOP+UV-treated cells showed that they were arrested in S and G2/M phases of the cell cycle, as would be expected if DNA replication were blocked (Figure 3F). The arrested cells had high levels of S345-phosphorylated Chk1, indicating activation of the DNA replication checkpoint pathway (Figure 3E). Similar results have been reported previously (Pichierri and Rosselli, 2004). When grown in culture, MOP+UV-treated HeLa cells did not proliferate while untreated cells grew with a doubling time close to 24 hours (Figure 3D). Cells exposed to long-wave UV light in the absence of MOP or cells incubated in MOP without UV exposure grew normally and showed little or no Chk1 phosphorylation.

Human embryonic stem cells (hESCs) require a feeder layer of mouse embryonic fibroblasts (MEFs) to grow in culture (Thomson et al., 1998). To determine whether psoralen-treated MEFs could be used as feeders, H7/WA07 hESCs were plated on a monolayer of primary MEFs that had been treated with 5 μ M AMT and irradiated with long-wave ultraviolet light for 30 seconds. After 5 days, healthy-appearing hESC colonies with smooth rounded borders were growing on the treated MEFs (Figure 4A). These colonies were indistinguishable from those grown on gamma-irradiated MEFs. The hESCs expressed normal amounts of the ES cell-specific transcription factor Oct3/4 (Figure 4B), indicating that they maintained their undifferentiated state. When plated on untreated MEFs, hESC colonies had indistinct borders suggesting the cells had differentiated prematurely. The ability of MEFs to support hESC growth declines with time (Villa-Diaz et al., 2009). We found that AMT+UV-treated MEFs would maintain hESCs in an undifferentiated state when plating was done within 6 days of treatment, but that after 8 days there was a noticeable decrease in the proportion of undifferentiated colonies (Figure S2).

The human keratinocyte precursor cell line CIN612 is widely used in studies of human papillomavirus (HPV) (Ozbun, 2002). CIN612 cells harbor episomal copies of HPV31b, a subtype that causes cervical cancer. The cells are typically grown on a feeder layer of mouse fibroblasts. Mouse J2 3T3 fibroblasts were treated with 5 μ M AMT and UV light for 2 minutes, then trypsinized and plated with CIN612 cells. After 3–7 days in culture, small colonies of CIN612 cells appeared that were indistinguishable from those grown on mitomycin C-treated feeder cells (Figure 4C, left). When untreated cells were used as feeders the colonies were much smaller (Figure 4C, right). The growth rate of CIN612 cells on psoralen-treated feeders was the same as on mitomycin-C treated feeders (not shown).

DISCUSSION

Treatment of cells with psoralen and ultraviolet light is a rapid, efficient, widely applicable, and nontoxic way to generate feeder layers for cell culture. The treated cells cease to proliferate but retain high viability for several days. Colonies of human embryonic stem cells (hESCs) and human keratinocytes precursors grown on psoralen-treated feeders are indistinguishable from those grown on gamma-irradiated or mitomycin-C treated cells. Psoralen treatment is faster, cheaper, and safer than gamma-irradiation or mitomycin C treatment. Because the irradiation time is short (30–60 seconds), psoralen enucleation could be easily adapted for the continuous in-line production of feeder cells on an industrial scale. The cells could be irradiated while flowing through an ultraviolet-transparent tube, and it would not be necessary to remove the psoralen afterwards as long as the cells were shielded from light. Psoralen treatment might also be useful for destroying the nucleus of one cell in cell fusion studies (Cowan et al., 2005), though the usefulness of this application is limited by the fact that the heterokaryon might not divide because of checkpoint activation. It may be possible to circumvent this problem by using cells that have genetic defects in checkpoint mechanisms.

Psoralen enucleation is also useful for enucleating certain types of recipient egg cells in nuclear transfer studies, such as opaque eggs that cannot be enucleated manually. The technique is especially suited for species with large eggs that undergo rapid cleavage divisions that are not regulated by cell cycle checkpoints, such as *Xenopus* and *Drosophila* (Raff and Glover, 1988). In these organisms, checkpoints do not become operational until the 12th cell division (Gerhart et al., 1984), by which time the psoralen-damaged DNA would have been segregated to a small group of cells. This group might also include common model organisms such as the zebrafish (Kane and Kimmel, 1993), *C. elegans*, and sea urchins (O'Farrell et al., 2004). The fertilized eggs of these species undergo rapid cleavage divisions but it has not been established whether checkpoints operate during early development. By contrast, in mammalian embryos checkpoints are operational during the early cleavage divisions (Liu et al., 2000b; Takai et al., 2000). Checkpoint activation can explain our observation that mouse eggs fertilized in vitro with psoralen-enucleated sperm cells do not undergo cleavage divisions. Because of checkpoint activation, psoralen-enucleated mammalian eggs are unlikely to be useful as recipients for nuclear transplantation. Manual enucleation is still the method of choice for mammalian somatic cell nuclear transfer.

Finally, psoralen enucleation provides an efficient means to generate either androgenetic or gynogenetic haploids for genetic studies in organisms that show extensive haploid development, such as *Xenopus* and zebrafish. These haploids are useful for determining if genetic traits are maternally inherited or imprinted.

EXPERIMENTAL PROCEDURES

Xenopus

Pigmented and albino frogs were purchased from Nasco. CMV-GFP(+) female frogs were the kind gift of Dr. Eddy M. DeRobertis and Douglas Geissert (UCLA Medical Center).

Antibodies

Anti-phospho S-345 Chk1 antibody was obtained from Cell Signaling Technology (#2341), and Oct4 antibody was obtained from Santa Cruz Biotechnology (#sc-9081).

Egg Enucleation

Freshly squeezed *Xenopus* eggs were incubated in a solution of 50 μ M AMT in 1X MMR (100 mM NaCl, 2 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 5 mM HEPES pH 7.4) for 5 minutes. During the incubation the eggs were rotated with forceps so that the white spot (indicating the position of the metaphase spindle) was facing upward. The eggs were then irradiated from above with a 100W ultraviolet light source (Zeiss HBO 100W/2) equipped with a D350/50X filter (Chroma). The lens of the light source was adjusted to give uniform UV intensity in the illuminated area, and the sample was placed 15.5 cm from the bulb. The beam intensity measured with a J221 long-wave UV meter (Ultraviolet Products, UVP) was approximately 15 mW/cm². The ultraviolet lamp was air-cooled with a small fan to prevent over-heating. Immediately after irradiation the eggs were fertilized and dejellied according to standard protocols (Murray, 1991) and allowed to develop in 0.1X MMR. Short-wave UV light was delivered with a UVGL-25 Mineralite (UVP) as described previously (Gurdon, 1960; Kroll and Gerhart, 1994).

Sperm Enucleation

A small piece of fresh *Xenopus* testis was macerated with forceps, resuspended in 1X MMR containing 1–50 μ M AMT, then filtered through a 35 μ M cell strainer (Falcon). The sperm suspension was spread onto a Petri dish then irradiated as described above and used immediately to fertilize fresh eggs. Mouse epididymal sperm were isolated in HTF medium (Quinn, 2000) and not filtered before irradiation.

Feeder Cell Enucleation

Cells were washed with Dulbecco's phosphate buffered saline (PBS) and incubated in PBS containing either 5 μ M AMT or 5 μ M MOP for 5 minutes. Cells were irradiated from above for 30–60 seconds with a 4 W UVGL-25 Mineralite (UVP) using the long-wave setting, holding the lamp as close as possible to the brim of the culture dish with the lid removed. After irradiation cells were washed with PBS. H7/WA07 hESCs (National Stem Cell Bank) were plated on primary MEFs 24 hours later. These MEFs were between passages 2 and 5. For keratinocytes precursor cell culture, the MEFs were trypsinized immediately after treatment and plated with CIN612 cells. Oct-3/4 antibodies were purchased from Santa Cruz Biotechnology.

Karyotype Analysis

Karyotype analysis was performed using a modified protocol from the Grainger laboratory (<http://faculty.virginia.edu/xtropicalis/KaryotypeXtropicalis.htm>). Briefly, stage 38–36 tailbud embryos were incubated in 0.1X MMR containing 10 μ M nocodazole for 1–3 hours. The tailbud was bluntly dissected off with sharp needles, incubated in 60% acetic acid for 5 minutes, and squashed onto a Superfrost Plus microscope slide (VWR Scientific) under a coverslip and a lead brick. After 5 minutes the slide was quick-frozen on a cake of dry ice, the coverslip was prized off with a razor blade, and the squashed tissue was stained with 0.1 μ g/

ml 4', 6-diamidino-2-phenylindole (DAPI). At least three high quality spreads were counted before assigning a karyotype.

Primary Cell Culture

Fertilized eggs were cultured in sterile $0.1 \times$ MMR containing penicillin and streptomycin. The medium was changed daily and the embryos were transferred to fresh dishes after hatching. When embryos reached stage 40, a group of 20 embryos was homogenized by pipetting up and down with a sterile 9 inch Pasteur pipette in 2 ml culture medium (70% Liebovitz medium containing 10% fetal bovine serum, glutamine, and penicillin-streptomycin). The homogenate was diluted to 20 ml with culture medium and plated on an 89 mm tissue culture dish. After 48 hours, tissue fragments were aspirated off and the adherent cells were cultured for a week, split 1:2, and then cultured for another week. Cells were trypsinized, stained with propidium iodide, and analyzed by flow cytometry according to standard protocols (Darzynkiewicz and Juan, 2001).

Nuclear Transplantation

Blastulae were dissociated into single cells by incubation in Dissociation Medium (2.5 mM NaHCO_3 , 7.5 mM Tris-HCl pH 7.6, 88 mM NaCl, 1 mM KCl, 0.5 mM EDTA) in an agarose-coated Petri dish. Recipient eggs were dejellied with 2% cysteine pH 7.8 then placed animal pole upwards in 1X MMR containing 25 μM MOP for 5 minutes then irradiated as described above for 5 minutes. Nuclear transfer was performed essentially as described by Gurdon (Gurdon, 1991).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Enucleation of Xenopus Eggs with Psoralen and Ultraviolet Light

(A) Experimental design for enucleating eggs.

(B) Top, appearance of typical diploid and haploid Xenopus embryos. Bottom, typical chromosome spreads from diploid and haploid embryos.

(C) Fresh Xenopus eggs were left untreated, irradiated with ultraviolet light for 5 minutes, incubated in 50 μ M AMT without irradiation, or irradiated in the presence of 50 μ M AMT. When the embryos reached the swimming tadpole stage, the percentage of embryos having a typical diploid, haploid, or abnormal appearance was determined.

(D) DNA content of primary cultured cells from control embryos, embryos derived from AMT +UV-treated eggs, and embryos derived from AMT-treated or UV-treated eggs.

(E) Eggs from a CMV-GFP female were left untreated or enucleated with AMT and UV light then fertilized with sperm. When the embryos reached stage 40–45 they were scored for GFP fluorescence.

(F) Left, eggs from a CMV-GFP(+) female were enucleated with AMT and UV light and transplanted with a GFP(–) blastula cell nucleus. Right, eggs from a GFP(–) female were enucleated with AMT and UV light then transplanted with a CMV-GFP(+) blastula cell nucleus.

Figure 2. Enucleation of Sperm cells with Psoralen + UV treatment

(A) Experimental design for enucleating sperm.

(B) Wild-type *Xenopus* sperm were treated with AMT+ UV light then used to fertilize eggs from an albino female. When controls reached stage 40–45, the percentage of embryos having a typical diploid, haploid, or abnormal appearance was scored.

(C) Typical embryos derived from the experiment described in part (B).

(D) *Xenopus* eggs or sperm were treated with different concentrations of AMT and irradiated for 5 minutes before fertilization. The number of haploid embryos was scored.

(E) *Xenopus* eggs or sperm were treated with 50 μ M AMT and irradiated for different times before fertilization. The number of haploid embryos was scored.

Figure 3. Psoralen + UV Treatment activates the DNA Replication Checkpoint

(A) Mouse eggs were left untreated or treated with 4–6 μM AMT and long-wave UV light for 30 seconds. They were parthenogenetically activated and four days later the extent of development was scored. Graph shows combined results from five independent experiments. *, $P < 0.05$ compared to control; **, $P < 0.01$; NS, not significant (Student's paired t-test).

(B) Mouse sperm were treated with 5 μM AMT, irradiated with a 100 W UV source for 15 seconds, and used to fertilize eggs in vitro. Arrows indicate the two pronuclei.

(C) HeLa cells were incubated in PBS containing different concentrations of MOP and irradiated for 1 min. After three days of culture the number of cells and the percent viability were determined.

(D) HeLa cells were left untreated or irradiated in PBS containing 5 μ M MOP for 1 min then transferred to culture medium. The number of cells and the percent viability were measured.

(E) HeLa cells were incubated in PBS containing 5 μ M MOP and irradiated with a 4W handheld long-wave UV source for 1 minute. At various times afterwards the amount of phosphorylated Chk1 was determined by immunoblotting. Arrowhead, phospho-Ser³⁴⁵ Chk1, asterisk, cross-reacting band that serves as a loading control.

(F) Exponentially growing HeLa cells were treated as in (E). After 18 hours of culture the DNA content was measured by flow cytometry.

Figure 4. Feeder Cells Prepared by Psoralen + UV Treatment

(A) A monolayer of primary MEFs was incubated in PBS containing 5 μ M AMT then irradiated for 30 sec (left) or left untreated (right). hESCs were plated on the cells and colonies were photographed after 6 days. Scale bar = 200 μ m.

(B) AMT + UV-treated cells were stained with antibodies to Oct3/4 and with Hoechst dye. Scale bar = 10 μ m

(C) CIN612 keratinocyte precursors were plated on untreated J2 fibroblasts (right) or fibroblasts that had been irradiated for 2 minutes in the presence of 5 μ M AMT. Scale bar = 100 μ m.

Table 1

Karyotype Analysis of Psoralen-treated and Control Embryos

Chromosome Number		
<u>Treatment</u>	<u>(Mean $\pm$ SD)</u>	<u># Embryos</u>
Control	36.0 $\pm$ 0.3	8
AMT+UV	18.0 $\pm$ 0.0	28
MOP+UV	18.0 $\pm$ 0.0	4
Short Wave UV	18.0 $\pm$ 0.0	3
Abnormal	35.4 $\pm$ 8.7*	13
Blastula SCNT	37.2 $\pm$ 3.5	9
Sperm AMT+UV	18.0 $\pm$ 0.0	27