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CHROMATIN INFLUENCE ON THE FUNCTION AND FORMATION OF THE NUCLEAR ENVELOPE SHOWN BY LASER-INDUCED PSORALEN PHOTOREACTION

SCOTT P. PETERSON AND MICHAEL W. BERNS
Developmental and Cell Biology, University of California, Irvine, Irvine, California 92717, *U.S.A.*

SUMMARY

Potorous tridactylis (PTK_2) cells growing in culture were treated with psoralen derivatives and dividing cells were located by phase-contrast microscopy. Psoralens, light-sensitive DNA-photoadducting drugs, were reacted with mitotic chromosomes through exposure to 365-nm light from an argon laser microbeam system. It was found that following mitosis and photoreaction, cells without nuclear envelopes were produced when psoralen-treated cells received 60 light pulses over their entire chromosome complement. These 'non-nuclear membrane' cells were found to incorporate [^{3}H]uridine and, to a lesser extent, [^{3}H]thymidine by autoradiography. Reduction of the light exposure by half (30 near-u.v. pulses) over the entire chromosome complement in the presence of psoralen also produced non-nuclear-membrane cells as seen by light microscopy. Further examination of these cells (30 light pulses) by single-cell electron microscopy revealed that unlike the high light exposure (60 near-u.v. pulses), the low light dosage resulted in cells with membrane patches associated with their chromatin. Since neither actinomycin D nor cycloheximide impeded nuclear envelope reformation, the psoralen-DNA reaction is concluded to produce non-nuclear-membrane cells by a mechanism other than transcription or translation inhibition. The association of Golgi with areas of nuclear membrane patches gives indirect evidence of a possible Golgi contribution to the reformation of the nuclear envelope after mitosis. It is concluded that DNA plays a role in envelope reformation.

INTRODUCTION

Selective irradiation or destruction of various cell organelles and metabolic pathways has been used to determine the function of various cellular systems. Laser microbeam experiments on chromosomes (Berns, Cheng, Floyd & Ohnuki, 1971), nucleoli (Sakharov & Voronkova, 1976) and centrioles (Berns, Rattner, Brenner & Meredith, 1977) have been useful in determining the normal function and relationships of these organelles to cell metabolism and growth. In addition, drugs, such as actinomycin D and cycloheximide, have been useful in blocking normal cell synthesis of RNA and proteins (Maul, Hsu, Borun & Maul, 1973) and in modifying organelle structure (Brinkley & Berns, 1974).

Recent work with nucleic acid photoreacting drugs (psoralens) has given information about the structure of DNA in chromatin (Cech & Pardue, 1977) and cellular

Address all correspondence to: Scott P. Peterson, Developmental and Cell Biology, University of California, Irvine, Irvine, California 92717, U.S.A.

DNA repair mechanisms (Ben-Hur & Elkind, 1974; Cole, Levitan & Sinden, 1976). Because of the photospecificity of these drugs for RNA and DNA, they could make excellent tools for the inactivation of entire chromosomes or specific parts of chromosomes when used in conjunction with a laser-near-u.v. microbeam system. One structure that is particularly amenable to study with this system is the nuclear envelope.

The chromatin associated with the nuclear envelope may consist of annuli and other fibrillar components (Engelhardt & Pusa, 1972; Scheer, Kartenbeck, Trendelenburg, Stadler & Franke, 1976; Schel & Wanka, 1973). Various authors have suggested that the chromatin material is involved with the initiation of DNA replication (Binkerd, Roach & Toliver, 1974) while others believe replication begins elsewhere in the nucleus (Wise & Prescott, 1973). In either case, there is a clear and very close structural and possibly functional relationship between the nuclear envelope and chromatin of the cell (Englehardt & Pusa, 1972).

The nuclear envelope is known to be associated with the endoplasmic reticulum (ER) in addition to the chromatin of cells. Mechanical injury to the nuclear envelope has been shown to be repaired by portions of the ER (Flickinger, 1970, 1974). Structural similarities between the nuclear envelope and the Golgi apparatus have also been shown (Franke & Scheer, 1972). Thus, a complex membrane system consisting of the nuclear envelope, ER and the Golgi exists in interphase eukaryotic cells (DeRobertis, Saez & DeRobertis, 1974).

Several theories of nuclear envelope reformation exist in the literature. These involve mitochondrial vesicles (Chai, Weisfeld & Sandburg, 1974), endoplasmic reticulum (Porter & Machado, 1960), membrane doublets (Szollosi, Callarco & Donohue, 1972) or other membrane elements (Murray, Murray & Pizzo, 1965; Robbins & Gonatas, 1964). All these mechanisms involve either a membrane element or vesicle though there is little agreement on the source of these membrane precursors. Experiments with protein synthesis inhibitors (emetine) and phospholipid synthesis inhibitors have not been successful in blocking the reformation of the nuclear envelope after mitosis (Maruta & Goldstein, 1975). These facts suggest that there is no *de novo* synthesis of nuclear envelope after cell division is complete.

In this work, we will show a relationship between the reformation of the nuclear envelope after mitosis and the chromatin. The psoralen derivatives AMT (4′-aminomethyl-4,5′,8-trimethylpsoralen), MMT (4′-methoxymethyl-4,5′,8-trimethylpsoralen) and HMT (4′-hydroxymethyl-4,5′,8-trimethylpsoralen) are to be used in conjunction with a laser microbeam system to photoreact mitotic chromosomes. The resulting cells are examined by autoradiography, light microscopy and single-cell electron microscopy.

MATERIALS AND METHODS

Cell culture

Potorous tridactylis, PTK_2 (American Type Culture Collection CCL no. 56), cells were grown in modified Eagle's medium (GIBCO) supplemented with 10% heat-inactivated foetal calf serum at 37 °C. Stocks were maintained in Falcon T-25 flasks and transferred to

Rose chambers for experiments. Cells were detached from growth surfaces with 0·125 % Viokase (GIBCO) and 0·1 % EDTA. This cell line was chosen for these experiments because it remains flat with the chromosomes visible during mitosis.

Drugs

Psoralen derivatives were generously provided by Dr John E. Hearst of UC Berkeley. AMT (4′-aminomethyl-4,5′,8-trimethylpsoralen) was dissolved in growth media at a concentration of 3·25 μg/ml. MMT (4′-methoxymethyl-4,5′,8-trimethylpsoralen) and HMT (4′-hydroxymethyl-4,5′,8-trimethylpsoralen) were both used at concentrations of 12 μg/ml (Isaacs, Shen, Hearst & Rappoport, 1977). Media containing psoralen were applied to cells 24 h before experiments were begun.

Psoralens require exposure to u.v. light of long wavelengths to react with nucleic acids (Weisehahn, Hyde & Hearst, 1977). For this reason, chambers were maintained in the dark and only observed with yellow light through the microscope.

Laser microirradiation

Selective exposure of chromosomes in dividing cells to near-u.v. light was accomplished through the use of a pulsed Hughes argon laser, model no. 3030H, operating at 365 nm and an intensity of 200 mJ/cm^2 per laser pulse measured by an International Photometer no. 600. A laser microbeam system (Berns, 1971) was used to focus the laser beam to a 5-μm^2 spot. Rose chambers containing a psoralen derivative in the growth media were placed on the microscope stage and mitotic cells located. Fifteen, 30 or 60 pulses of laser light were applied to different numbers of chromosomes in late prophase, prometaphase, or metaphase cells.

Autoradiography

At different times after irradiation, [^{3}H]uridine or [^{3}H]thymidine were added to the Rose chambers. Both nucleosides were used at activities of 10 μCi/ml in 2 ml of media. Cells were pulsed with [^{3}H]uridine for 40 min and [^{3}H]thymidine for 2 h, then rinsed in phosphate-buffered saline containing non-radioactive nucleosides. Cells were fixed in 0·1 % glutaraldehyde in Hanks' Basic Salt Solution overnight at 4 °C. The coverslips were air dried for 8 h, glued to microscope slides, and air dried overnight. Autoradiography was performed with Kodak NTB-3 emulsion diluted 1:2 with distilled water. [^{3}H]uridine samples were allowed to expose for 3 weeks and [^{3}H]thymidine for 4 days at 4 °C. Autoradiograms were developed in Kodak D-19 for 5 min, stained with aceto-orcein, dehydrated in ethanol with 2 final washes in xylene, and a top coverslip mounted with Permount.

Electron microscopy

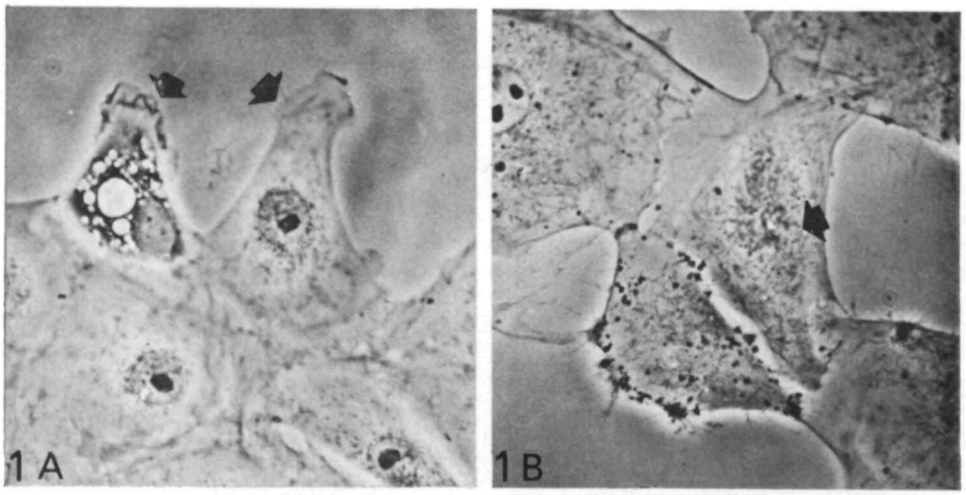
Cells were prepared for single-cell EM as has been previously reported (Rattner & Berns, 1974). Epon-embedded cells (8·3 ml nadic methyl anhydride, 9·9 ml dodecyl succinic anhydride, 18 ml of Epon 812 and 1 ml DMP-30) were sectioned with a glass knife on an LKB Ultratome III. Sections were viewed on a Siemens 1A at 60 kV.

Light microscopy

Light microscopy was performed using a Zeiss Photomicroscope II fitted with phase-contrast optics and a yellow filter.

RESULTS

Table 1 and Fig. 1 show the effect of photoadduction of psoralen to mitotic chromosomes. Similar results were obtained for all the psoralen derivatives tested here. In all the studies reported here, it appears that the effect on nuclear envelope reformation is dependent on the amount of chromosomal material irradiated (i.e. individual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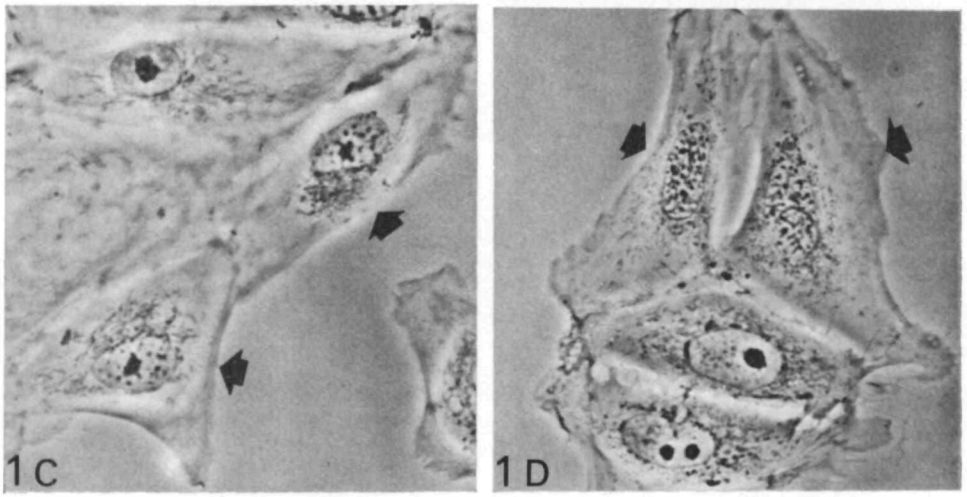
1 B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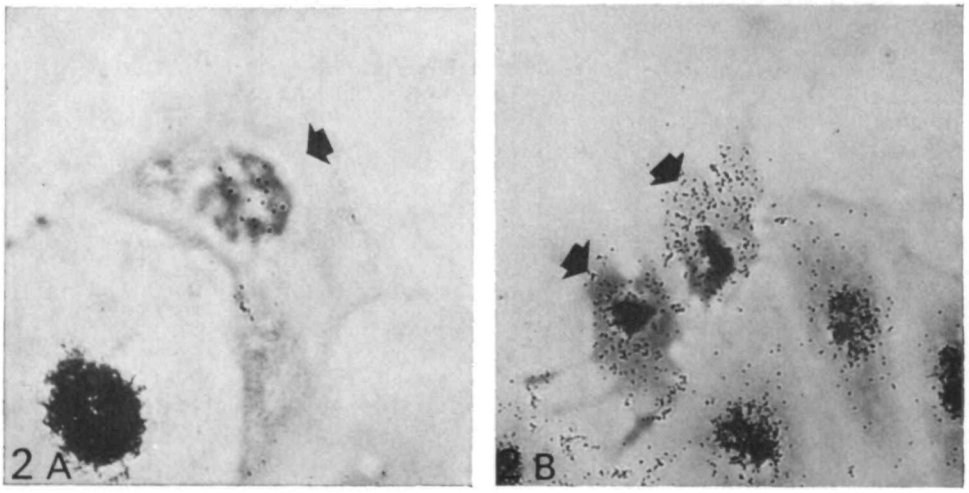
2 A
B

chromosomes versus the entire chromosome complement) and the amount of near-u.v. light the cells are exposed to. Irradiation of various single chromosomes or chromatids (Table 1) does not stop the reformation of the nuclear envelope although nuclei may become pycnotic and have micronucleoli, 20/36 cases (Fig. 1 A). Inhibition of nuclear membrane formation is seen by light microscopy at higher (30–60 near-u.v. pulses)

Table 1. *Effects* of psoralen photoaddition on the reformation of the nuclear envelope (u.v. wavelength used 365 nm)*

No. of chromosomes irradiated	60 u.v. pulses alone	15 u.v. pulses + psoralen	30 u.v. pulses + psoralen	60 u.v. pulses + psoralen
1 chromatid	0/15	0/15	no data	0/15
1 chromosome	0/12	0/10	no data	0/17
¼ of metaphase plate	0/12	no data	0/12	0/18
½ of metaphase plate	0/15	no data	1/15	3/18
All chromosomes of cell	0/23	0/15	28/38	25/27

* Fraction represents the no. of cells without a nuclear envelope over the total no. of cells examined. All above data assayed by light microscopy 24 h after irradiation. Cells giving rise to 2 non-nuclear-membrane daughters are counted as one cell.

laser exposures, primarily when the entire chromosome complement of the cell is photoreacted (Fig. 1 B). In control experiments, exposure of the entire chromosome set to 365-nm laser light alone does not interfere with the reformation of the nuclear envelope. It should also be noted that 100 μg/ml cycloheximide or 2 μg/ml actinomycin D had no effect on the reformation of the nuclear envelope when added 30 min before the onset of division although other abnormalities, such as micronucleoli and vacuolation, were seen (Fig. 1 C, D). These inhibitor concentrations are enough to reduce normal incorporation of [^{14}C]alanine and [^{3}H]uridine by 98% as measured

Fig. 1. A, single chromatid photoreacted in metaphase by laser-AMT bonding, 24 h after irradiation. Arrows indicate daughter cells from division. Note presence of micronucleoli and vacuoles. × 1000.

B, 11 chromosomes of cell photoreacted during early metaphase with 60 near-u.v.-laser pulses. Note lack of normal nucleus and denser chromatin mass (arrow) in the centre of the cell. × 1100.

C, 100 μg/ml cycloheximide in media. Arrows indicate cells from mitotic division which began 30 min after the addition of inhibitor. × 1000.

D, 2 μg/ml actinomycin D added to media. Arrows indicate daughter cells from mitotic division which began 40 min after addition of transcription inhibitor. Note presence of nuclear envelope and lack of normal nucleolus. × 1200.

Fig. 2. A, [^{3}H]thymidine incorporation in non-membrane cell (arrow) 8 h after treatment with 60 near-u.v. pulses in the presence of psoralen. Other cell shows normal incorporation due to DNA replication. × 1000.

B, [^{3}H]uridine incorporation in non-membrane cell 24 h after treatment with 60 near-u.v. pulses in the presence of psoralen (arrows). × 900.

by TCA precipitation and liquid scintillation counting (Maul *et al.* 1973). Light-microscopic observations of the psoralen-laser cells without nuclear membranes show they are capable of maintaining themselves with few vacuoles or granules for periods exceeding 48 h. Cells without nuclear membranes may be shown to be viable for this period by dye (trypan blue) exclusion.

A cell may or may not undergo cytokinesis or karyokinesis after laser-near-u.v. psoralen treatment; Fig. 1B is a typical cell that underwent cytokinesis. This seems to be dependent on the stage of mitosis at which the irradiation occurs. Prophase cells give rise to single-cell products 80% (10/12) of the time while metaphase cells produce pairs of daughters in 90% (14/15) of the cases examined. In either case, no nuclear envelope forms around the chromatin of these cells. None of the 'non-membrane' cells were seen to undergo any subsequent cell divisions even though they were followed for periods up to 56 h after irradiation.

Table 2. *Ability of treated cells to incorporate nucleosides*

	Condition of nucleoside addition	8 h post-irradiation	24 h post-irradiation
Non-membrane cell	[^{3}H]uridine	1/19	22/77
Non-membrane cell	[^{3}H]uridine + 0·6 μg/ml Act. D	—	0/20
Non-membrane cell	[^{3}H]thymidine	11/23	0/19
Cell treated with laser alone over all chromosomes	[^{3}H]uridine	16/16	18/18
Cell treated with 3·25 μg/ml AMT alone	[^{3}H]uridine	—	15/15

Non-membrane cells were prepared by irradiation with 60 pulses of 365-nm laser light over entire chromosome complement in presence of 3·25 μg/ml AMT. Fraction represents the no. of cells showing nucleoside incorporation as assayed by autoradiography.

Cells lacking a nuclear envelope are capable of the uptake of tritiated nucleosides (Table 2, Figs. 2, 3). [^{3}H]uridine is readily incorporated and is found throughout the cell indicating that an intact nuclear envelope is not required for transcription in eukaryotic cells (Fig. 2B). Whether this is normal transcription or uncontrolled transcription cannot be determined. The appearance of grains over these cells cannot be attributed to cytoplasmic accumulation since 0·6 μg/ml actinomycin D will stop [^{3}H]uridine incorporation. [^{3}H]thymidine is also accumulated in non-membrane cells (Fig. 2A). Only a very few grains are found over the central chromatin mass in these cells. These grains could represent the repair of psoralen-DNA photoadducts by excision repair mechanisms (Ben-Hur & Elkind, 1974; Cole *et al.* 1976). The uptake of nucleosides occurs at different times after the non-membrane cell is produced (Table 2). Incorporation of thymidine occurs most readily in the first 8 h after irradiation while transcription (uridine uptake) occurs 16–24 h later.

Electron-microscope examination of non-nuclear-membrane cells (treated with 60 near-u.v. pulses and psoralen over the entire chromosome complement) shows

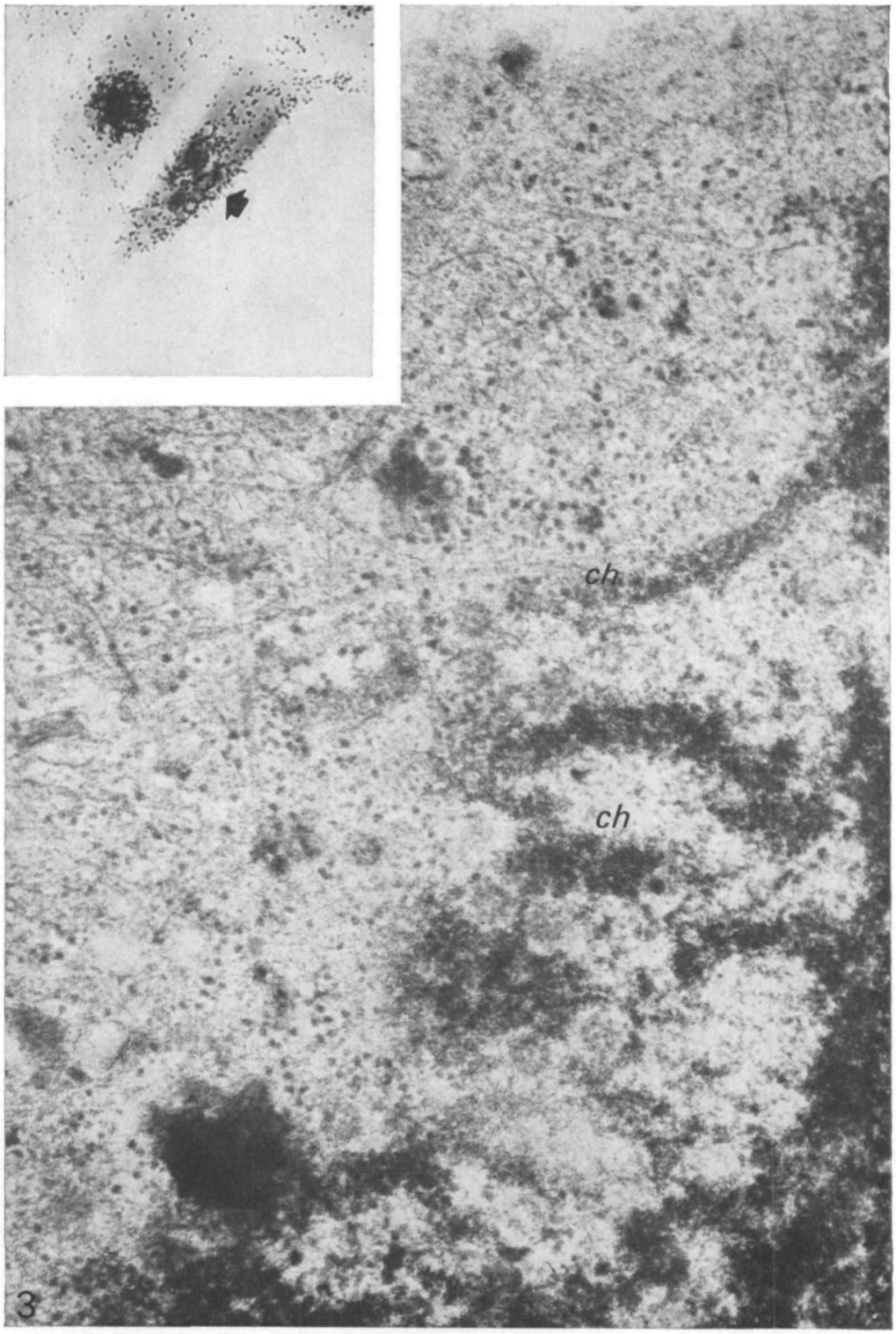

Fig. 3. Single cell electron microscopy of a cell that showed incorporation of [^{3}H]-uridine (inset: arrow). After autoradiography, the photographic emulsion was removed by heating the coverslip in 47 °C phosphate-buffered saline for 40 min. Normal preparation for EM was then followed. *ch*, chromatin. Note lack of normal nuclear envelope as illustrated in Fig. 5. Inset, ×900; micrograph, ×30000.

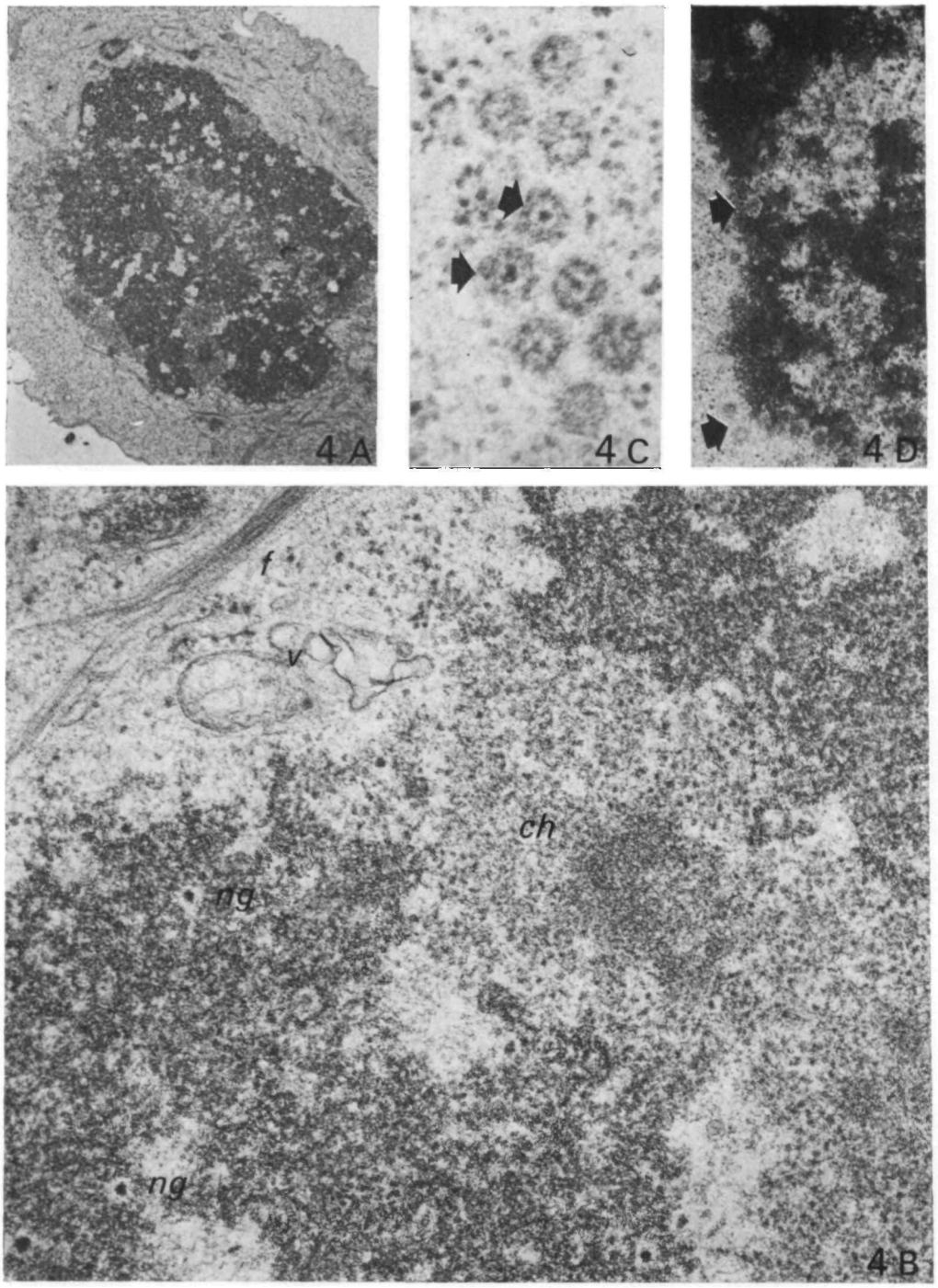

Fig. 4. A, electron micrograph of non-membrane cell 48 h after treatment with 60 near-u.v. pulses in the presence of psoralen. Note lack of apparent nuclear envelope around central chromatin mass. × 6000.

B, higher magnification picture of the edge of chromatin mass showing absence of nuclear envelope. *ch*, chromatin; *f*, microfilaments; *ng*, nucleolar granule from breakdown of nucleolus; *v*, vesicle. × 25000.

C, annular rings (arrows), normally found in the envelope, free in the chromatin of non-membrane cell. × 60000.

D, annular rings (arrows) associated with chromatin in non-nuclear membrane cell. × 18000.

that they are without any apparent membrane around their chromatin (Figs. 3, 4) when compared with untreated interphase cells (Fig. 5). Usually these cells consist of a central chromatin mass which is very thick and dense with a few diffuse areas. Non-nuclear-membrane cells have chromatin that is apparently less condensed than mitotic chromosomes but denser than normal interphase chromatin. Small membranous vesicles are often seen near the edge of the chromatin mass (Fig. 4B). Annuli, normally found in the nuclear pore (Fig. 5B), are seen around the edges of the chromatin (Fig. 4C, D). Granules of approximately 30 nm diameter are seen throughout the chromatin mass and may be breakdown products of the nucleolus as has been previously reported (Brinkley, 1965).

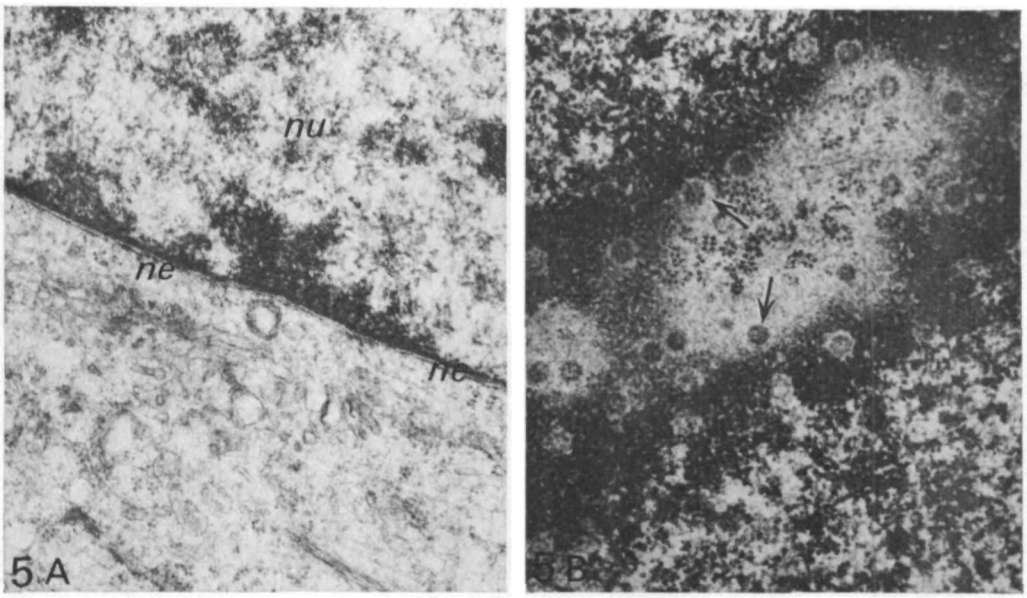

Fig. 5. A, cross-section through edge of normal nucleus (*nu*) showing bilayer of nuclear envelope (*ne*). Note appearance of chromatin and envelope. × 20500.
B, tangential section through the nuclear envelope in untreated PTK_2 cell. Arrows indicate annuli of the nuclear pore. × 24000.

When the u.v.-laser light exposure is reduced by half (30 pulses in the presence of psoralen), there is still no apparent nuclear envelope seen in treated mitotic cells by light microscopy: upon examination by single-cell EM techniques the reduced 365-nm exposure allows the formation of membrane patches around the central chromatin mass (Fig. 6). These patches are often associated with short lengths of ER. Near the edge of the chromatin mass, the patches become attached to regions of the chromatin (Figs. 6, 7) but not to the extent of untreated cells (Fig. 5). Membrane pieces seemingly separated from the central chromatin mass also have electron-dense, chromatin-like material associated with them (Fig. 7). In some areas, there are several layers of reforming nuclear envelope material (Fig. 7). These cells (thirty 365-nm pulses) also lack the free annuli seen in cells treated with 60 near-u.v. pulses in the presence of psoralen.

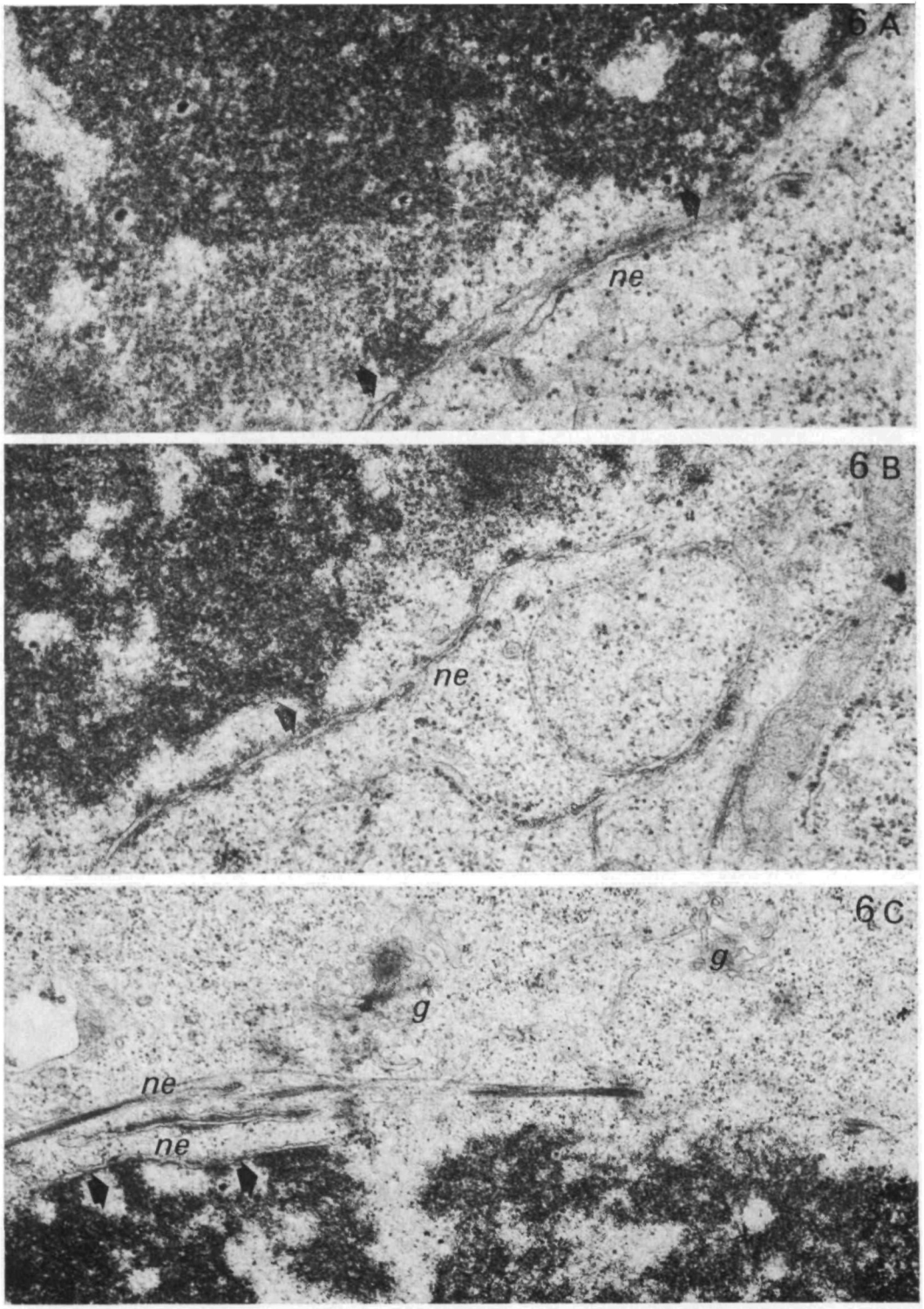

Fig. 6. Various areas of partially reformed nuclear envelope in cells treated with 30 near-u.v. pulses. All micrographs were taken from cells fixed 24–48 h after photoadduction with psoralen. Note association of chromatin with membrane patches at arrows. *g*, Golgi; *ne*, nuclear envelope. A, ×35000; B, ×30000; C, ×20000.

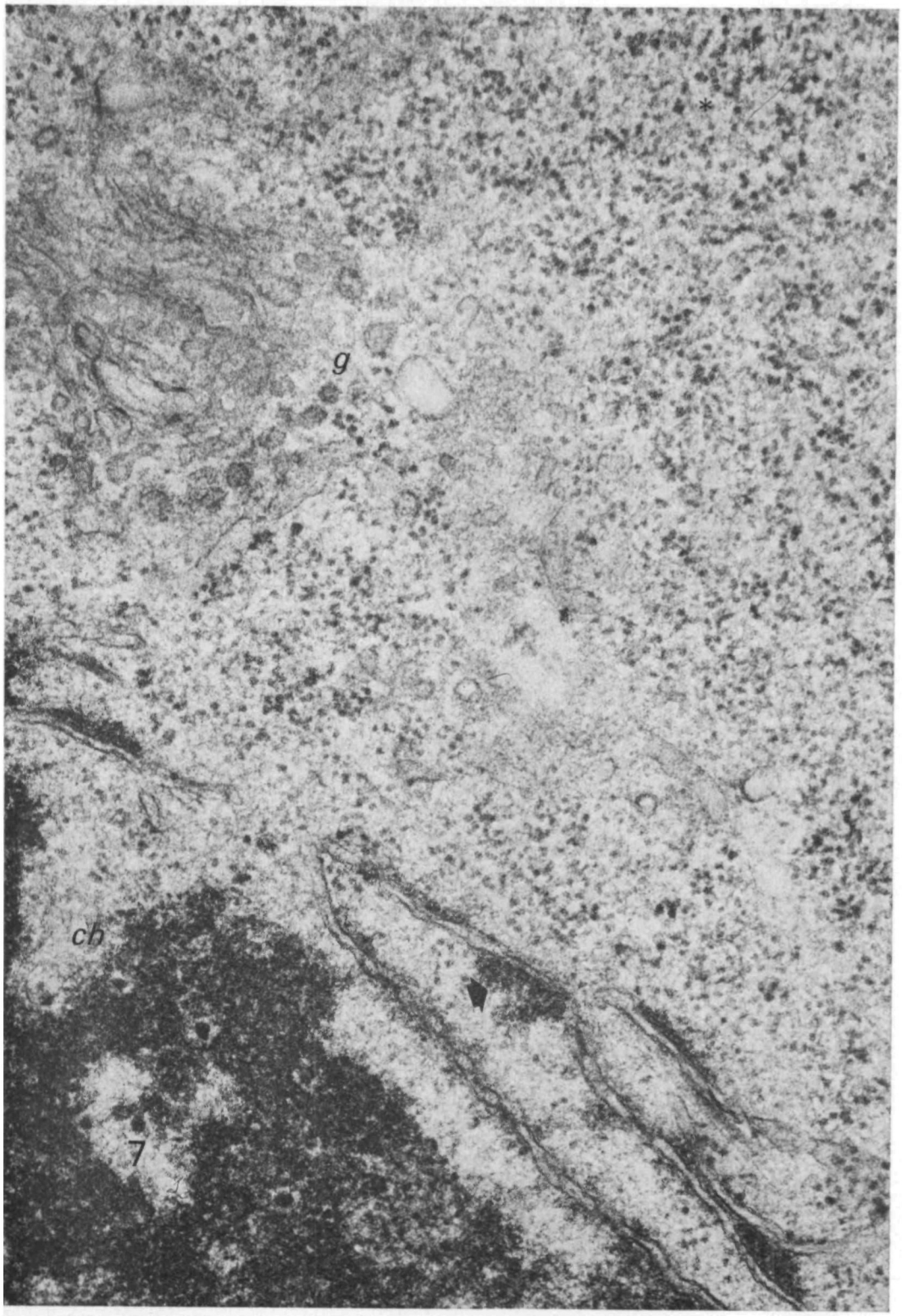

Fig. 7. Golgi and reforming nuclear envelope in cell fixed 48 h after treatment with 30 near-u.v. pulses in the presence of psoralen. Note apparent chromatin association with membrane (arrow) and the presence of Golgi vesicles (*g*) in cytoplasm. *ch*, chromatin. × 40000.

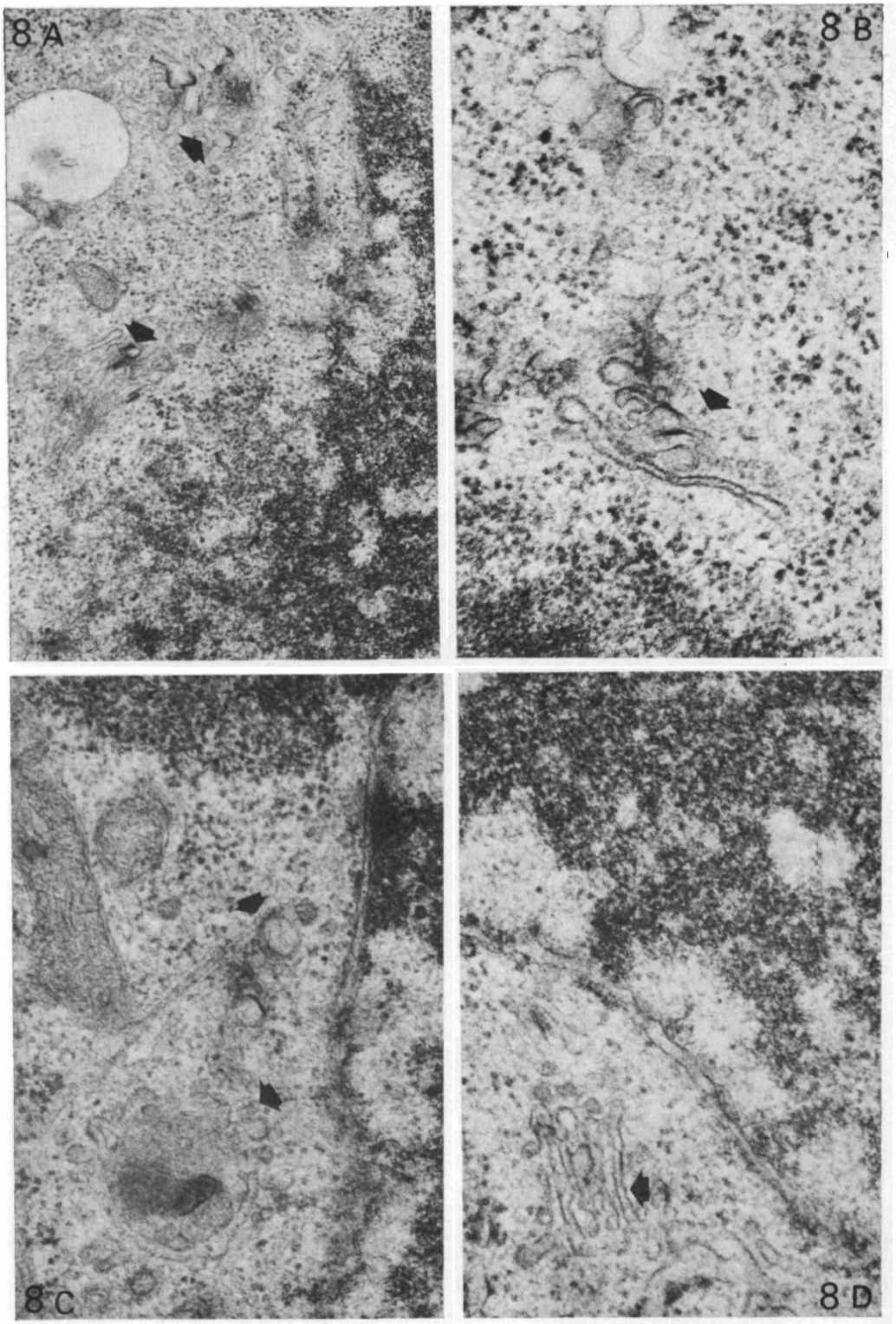

Fig. 8. Various areas of reforming nuclear envelope associated with Golgi and vesicular elements (arrows). All cells treated with 30 near-u.v. pulses and psoralen. A, ×15000; B, ×22000; C, ×23000; D, ×25000.

Frequently, the patchy membrane areas are associated with the Golgi apparatus (Figs. 7, 8). Small vesicles may be seen merging with or budding off the Golgi and dispersing into the cytoplasm in the region of the chromatin mass. Rarely, Golgi can be seen abutting on the chromatin with no mediating nuclear envelope separating the two.

DISCUSSION

Photoaddition of psoralens to mitotic chromosomes has a definite effect on dividing cells. Non-nuclear-membrane cells are produced which show highly condensed chromatin, large amounts of Golgi and a very flat morphology In spite of these alterations, cells are still able to conduct some metabolic functions, such as transcription and DNA repair or replication.

Cells without nuclear envelopes are capable of uridine uptake and unscheduled DNA synthesis. The approximate 30% of the cells incorporating uridine (Table 2) is lower than the expected 95–100% in untreated cells. This is probably due to the effects of DNA photoadduction by the psoralen. The higher than normal frequency (50% of the cells tested) of incorporation of [^{3}H]thymidine may also be attributed to the effects of DNA photoreaction.

In light of *in vitro* transcription work, it is probably not surprising that cells without a nuclear envelope can transcribe. The experiments presented here, however, say nothing about the RNA transcription represented by the uridine uptake. Transcription in non-nuclear membrane cells may be very different from that seen in normal, non-treated interphase cells.

The presence of transcription (uridine uptake) and DNA synthesis is not important to the reformation of the nuclear envelope. Previous work has shown that the nuclear envelope can reform in a variety of inhibitors (Maruta & Goldstein, 1975; Seider-Blum & Burger, 1977) including the cycloheximide and actinomycin D examined in this work. *De novo* synthesis, therefore, is not required for the reformation of the nuclear envelope in mitotic cells. The blockage of nuclear envelope reformation by photoadducting DNA with psoralens probably is not due to inhibition of transcription or translation. Some other mechanism must be responsible for interfering with nuclear envelope reformation.

The action of psoralens seems to be on those areas of chromatin unprotected by proteins (Weisehahn *et al.* 1977; Hanson, Shen & Hearst, 1976; Cech & Pardue, 1977). Work by Hanson *et al.* (1976) has shown that psoralen crosslinks occur every 200 base pairs which is the approximate number of base pairs found between adjacent histone nucleosomes (Lohr, Tatchell, Kovacic & Van Holde, 1977). Nucleosomes (nu bodies) have been shown to exist in metaphase chromosomes (Rattner, Branch & Hamkalo, 1975) so the non-nuclear-membrane effects in this work can be attributed to psoralen bonding in the inter-nu body regions, not to bonding in areas of DNA associated with histone proteins. This conclusion follows based upon the above mentioned work of Hanson *et al.* (1976).

The probability of psoralen-DNA-protein complexes or psoralen-protein complexes

is low. The normal reaction of psoralen with nucleic acids in the presence of near-u.v. light is through the formation of cyclobutane structures between the 5,6 double-bonded carbons of pyrimidines with the 3,4 or 4′,5′ double-bonded carbons of the psoralen (Musajo *et al.* 1967; Weisehahn *et al.* 1977). This reaction is begun by the intercalation of the psoralen molecule into double-stranded nucleic acids (Weisehahn *et al.* 1977). The exposure of the intercalated molecules to near-u.v. light causes the reaction between pyrimidines and psoralen which may produce covalent interstrand crosslinks and monoadducts. Accepting this mechanism for psoralen u.v. action would not allow the formation of psoralen-DNA-protein complexes. Previous work has shown that general binding of psoralens to cellular proteins does not occur (Peterson, unpublished results). The specificity of psoralens for nucleic acids and the reacting site of psoralen both indicate that the non-membrane cells are a result of altered DNA and not of protein interactions with psoralen.

The formation of non-nuclear membrane cells is dependent on the amount of near-u.v. light psoralen-treated cells are exposed to. By increasing the dose of 365-nm laser light, the number of psoralen photoadducts and crosslinks to the DNA of the cells is increased (Johnston, Johnson, Moore & Hearst, 1977). The appearance of non-nuclear-membrane cells is directly correlated with increasing light exposure. High near-u.v. doses result in non-membrane cells (sixty 365-nm laser pulses), moderate exposures (thirty 365-nm laser pulses) result in cells with small membrane patches, and low near-u.v. exposures (15 pulses) do not affect nuclear envelope reformation. Since increasing the u.v. exposure increases the number of psoralen interstrand crosslinks and photoadducts (Johnston *et al.* 1977; Weisehahn *et al.* 1977), mitotic cells must require that a certain amount of DNA be damaged to effectively block nuclear envelope reformation. The effect is graded; by altering the number of photoadducts, the amount of interference with membrane formation is also changed. A general pattern seems to be that the more photoreacted the cell is, the less nuclear membrane will be formed after cell division.

The random irradiation of single chromosomes and various numbers of chromosomes along the metaphase plate does not rule out the possibility that a certain set or number of chromosomes may direct nuclear envelope reformation. The few non-nuclear cells produced when half or three-quarters of the metaphase plate was irradiated may be due to the photoadduction of the correct chromosome set. However, other data by Berns *et al.* (1972) has shown that micronucleoli can be formed around chromosomes randomly deleted from the mitotic apparatus. Thus, photoreaction of a certain chromosomal set is probably not vital to envelope reformation since the ability to direct envelope reorganization seems inherent in all chromosomes. The non-nuclear effect is most probably due to interference with DNA in the entire chromosome complement.

Chromatin always seems to be associated with the nuclear envelope (Comings & Okada, 1970; Troncale, Bass, Daly & Goto, 1972). In cells with patchy membrane units, chromatin is always seen associated with the side of the membrane facing the chromatin mass. In normal cells, some workers have noted the presence of chromatin connections to annuli (Troncale *et al.* 1972; Engelhardt & Pusa, 1972). In this study,

we have shown that the close interrelationship of the membrane and chromatin in the nucleus is altered by psoralen photoadducts. Annuli are found free along the edge of the chromatin instead of embedded in the nuclear membrane which has been eliminated by psoralen treatment. Cells with the greatest near-u.v. exposures and the greatest number of psoralen photoadducts are those with the least amount of membrane. It seems reasonable to conclude that the DNA of the chromatin is involved in the normal reconstitution of the nuclear envelope and that psoralen blocks this function.

The Golgi may be considered part of the endoplasmic reticulum (DeRobertis *et al.* 1974). Other authors have shown structural similarity between the Golgi and the nuclear envelope (Franke & Scheer, 1972). In this work, the Golgi is often seen along the edge of the chromatin mass in the area of reforming nuclear membrane patches. This is not conclusive evidence of a Golgi contribution to nuclear envelope formation or repair, but it is consistent with other theories of nuclear envelope formation (Porter & Machado, 1960; Chai *et al.* 1974; Szollosi *et al.* 1972; Murray *et al.* 1965; Robbins & Gonatas, 1964). Vesicular elements, possibly budding off the Golgi, in the area of the nuclear membrane patches may be the same as those seen by other authors.

Psoralens may impede the reformation of the nuclear membrane by many mechanisms. One may be that the photoadduction of the chromosome does not allow the decondensation after mitosis needed to expose structural chromatin elements involved in membrane formation. It could also be that the photoreacted DNA (between the nu bodies) stops the normal association of DNA with the forming nuclear envelope.

Work by Maul (1977) has shown that membrane formation begins in early telophase when the chromatin is still highly condensed. Such data indicate that the degree of chromatin density is not a determining factor in nuclear envelope reformation. In the patchy-membrane cells, the appearance of membrane units seems independent of the amount of decondensation – they are equally prevalent in diffuse or dense chromatin areas. The orientation of DNA-nuclear-membrane components may be a more important factor than general condensation in organizing membrane reformation.

Digestion of isolated nuclear envelope with DNase *in vitro* has suggested that DNA is an important structural component of the nuclear envelope (Agutter, 1972). In this study, we have shown that photoadduction of DNA will prevent the reformation of the nuclear envelope. Unlike digestion studies, the DNA is not destroyed but exists in an altered conformational state. Our results suggest that the DNA elements needed for nuclear envelope reformation must not only be intact but exist in the correct configuration for normal nuclear envelope reformation and structure.

Obviously, the effect of psoralen bonding and how it impedes nuclear membrane formation are very complex. The mechanisms offered here for interference with membrane formation are based on the site of psoralen action at the molecular level. Several points can be made about the psoralen-produced non-membrane cells: (1) psoralen photoadduction of DNA does inhibit reformation of the nuclear envelope after division; (2) lowering the amount of psoralen photoadduction produces partial

nuclear membranes; (3) nuclear membranes seem to form from vesicular units; (4) the Golgi may contribute material to the reforming nuclear membrane; and (5) loss of the nuclear membrane does not stop transcription or DNA synthesis.

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