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*Invited Review*

# Cell rounding with «Rip off» detachment

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**Summary.** The «rounding up» characteristic of mitotic, apoptotic, transformed and non-transformed cells appears to be initiated by various causative factors. Cytoskeletal and adhesion modulations have been implicated. Endocytic internalization via large channels seen in the rounding of amoeba and human cells alike, implicate a third mechanism, viz. membrane flow and fusion, where «rip off» detachment sheds parts of the cell to achieve freedom. Mutilation as a means for detachment is also used by moving fibroblasts, linking shape change with movement via a distinct mechanism. While rounding may follow detachment and detachment may follow rounding, «rip off» mutilation, whether self-inflicted or imposed, and modulation of stickiness are fundamentally different processes.

**Key words:** Surface area, Large channel endocytosis, Adhesion, Cytoskeleton, Apoptosis

## Introduction

The «well-spread», flattened morphology of anchorage-dependent cells undergoes a dramatic flat-to-round (FTR) change in the transition from the interphase period into the M-phase period of each cell cycle, both in-vitro and in-vivo (Zeligs and Wollman, 1979). The morphological change is reversed into round-to-flat (RTF), on exiting M-phase to return to interphase. Alternations of FTR and RTF morphological changes characterize normal, non-tumorigenic «anchorage-dependent cell growth» while the high proliferative cancer states are characterized by «anchorage-independent cell growth» where the FTR and RTF morphological changes are less well delineated (Wright et al., 1977; Kahn and Shin, 1979). Although cell spreading is crucial to cell proliferation in anchorage-dependent normal, non-tumorigenic cells (Folkman and Moscona, 1979) it appears to be not factored into anchorage-binding or adhesion but rather into the extent to which the cell could spread out its surface, since an engineered increase in substrate adhesiveness by even a thousand folds produces no effect so long as the cell is prevented from enlarging its surface area (Singhvi et al., 1994). In the malignant phenotypes the ability to metastasize is seen correlated with losing the sprawling «well-spread» morphology and becoming rounded (Raz and Ben-Ze'ev, 1983). That being the case, understanding the basis of rounding and its modulation would have therapeutic impact on metastatic dissemination which is the most common cause of fatalities in cancer. However a fundamental linkage between cell rounding and movement which is needed for migration appears unclear although shape and motility have long been recognised as two interdependent features (Haemmerli and Felix, 1982).

«Rounding up» is also recognised as «stereotypical» and the «cardinal» morphological characteristic of suicidal cells in apoptosis or programmed cell death which have no proliferative capability (Wyllie et al., 1980; Kerr and Harmon, 1991; Sit et al., 1994a). Apoptotic cells are now recognised as having central importance in development, tissue homeostasis and an array of the most serious of human afflictions, viz., cancer, autoimmune disorders, AIDS, neurodegenerative diseases, ischaemic and toxic injuries (Thompson, 1995; Ashwell et al., 1994). In more fundamental cell life processes, the rounding shape change has long been associated with endocytosis and membrane flow in the amoeba (Stockem and Wohlfarth-Bottermann, 1969) as well as in embryonic morphogenetic cell movements which are relevant to the establishment of cell lineages (Keller and Hardin, 1987).

The «rounding up» characteristic is not always just a shape change. Concomitant downsizing of the cell, increase in cell density, reduction in plasma membrane surface area, development of numerous long anchoring microvilli or filopodia, and detachment have been observed (Sit and Wong, 1989; Sit et al., 1991b, 1993a,

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**Table 1.** Cytoskeleton changes.

| ROUNDING MEDIATOR | CELL TYPE | REFERENCE |
|---|---|---|
| Disassembly of actin cytoskeleton via phosphorylation by serum growth factor, lysophosphatidic acid, thrombin receptor-activating peptide, phospholipase D, p60src protein tyrosine kinase, and Rho subfamily of small GTPases. Pervanadate inhibits rounding | Dictyostelium N1E-115 and NG108-15 neuronal cells, vero cells. | Chardin et al., 1989; Geiger et al., 1992; Jalink and Moolenar, 1992; Howard et al., 1993; Jalink et al., 1993; Nobles and Hall, 1994 |
| Cytoskeletal hyperphosphorylation in serine/threonine residues by okadaic acid (inhibitor of protein phosphatases) causing rounding. | Hepatocytes. | Reaven et al., 1993. |
| Platelet-derived growth factor (PDGF) and phorbol 12-myristate 13-acetate (PMA) causing rounding with disruption of stress fibres. | IIC9 fibroblasts. | Ha and Exton, 1993 |
| Epidermal growth factor (EGF) induced phosphorylation of ezrin cytoskeletal protein causes rounding but EGF-induced phosphorylation of spectrin cytoskeletal protein causes flattening. In mitotic cells, spectrin phosphorylation is seen as causing detachment and rounding. | A431, CHO and HELA cells. | Bretscher, 1989; Fowler and Adam, 1992; Suarez-Quian and Byers, 1993. |
| Vimentin intermediate filaments phosphorylation caused by withangulatin A, associated with redistribution of heat-shock protein HSP72. | Brain tumor cells. | Lee et al., 1993. |
| Phosphorylation of the 20 kDa myosin light chain induced by Concanavalin A and fetal calf serum. | Smooth muscle cells. | Sasaki and Sasaki, 1990. |
| Hydrostatic pressure causing rounding with disruption of cytoskeletal elements. | MG-63 osteosarcoma cells. | Haskin et al., 1993. |
| Epidermal growth factor-induced cytoskeletal changes, modulated by microgravity. | A431 epidermoid carcinoma of skin. | Chinkers et al., 1981; Rijken et al., 1991. |
| Calcium loading and toxic cell death, via ionophore A23187, membrane damage, phospholipase C and sodium cyanide treatment, causing actin microfilament contraction | Ventricular myocytes, smooth muscles cells, hepatocytes. | Schanne et al., 1979; Albrecht et al., 1983; Hayashi et al., 1990. |
| Oxidation and cross-linking of actin microfilaments by N-methylformamide and hydrogen peroxide. | Primary fibroblasts, melanoma cells (M14), pulmonary endothelial cells. | Malorni et al., 1992; Siflinger-Birnboim et al., 1992. |
| Actin microfilament and stress fibre disruption by Herpes simplex virus and *Clostridium difficile* toxins. | Kidney cells, HEp-2 cells, and T84 intestinal epithelial cells. | Falke et al., 1981; Fiorentini et al., 1989; Hecht et al., 1992. |
| Stabilization of microtubule polymerization by taxol enhances ACTH-induced rounding. | Adrenocortical tumor cells | Benis and Mattson, 1989. |
| Trypsin-induced rounding inhibited by stabilization of microtubule polymerization by taxol. | Neural NG108-15 and CHO cells. | Smalheiser, 1991. |
| Mitotic poisoning by antitubulin agents, viz. colchicine and taxane. | Cell lines | Margolis and Wilson, 1977; Rowinsky and Donehower, 1992; Hamell et al., 1995 |
| Microtubule reorganization and depolymerization caused by *Autographa californica* M nuclear polyhedrosis virus. | *Spodoptera fruigiperda* IPLB-Sf-21 cells. | Volkman and Zaal, 1990. |

1994a). This review will consider the various causations of rounding and draw attention to the contribution by plasma membrane cycling where modulation of membrane fusion and «rip off» is of central importance, both in cell movement and in rounding with detachment.

## Cytoskeletal changes

The concept of a «cytoskeleton» has been evolved to explain how cell shape is maintained and altered during such processes as endocytosis/exocytosis, mitotic cytokinesis, and amoeboid movements involving sol-gel conversions of cytoskeletal components (Janson and Taylor, 1993). There are 3 main components in the cytoskeleton, viz. (1) microfilamentous and stress fibre actin, and non-filamentous actin (Cao et al., 1993), (2) intermediate-sized filaments (keratin or tonofilaments, desmin, vimentin, neurofilaments and glial filaments (glial fibrilar acidic proteins)), and (3) microtubules and microtubule-associated proteins (MAPs) that have a crucial role in the regulation of microtubule polymerization. Table 1 shows that cell rounding can be mediated by any of the 3 cytoskeletal components, viz., (a) actin microfilament and stress fibre disruption mediated by phosphorylation, $Ca^{2+}$-loading, mechanical, oxidation, and toxic injury; (b) phosphorylation of vimentin intermediate filament network that is dependent on microtubule morphology; and (c) microtubule depolymerization by cold treatment, toxic injury and anti-tubulin mitotic poisons (microtubule poisons), e.g., colchicine, colcemid, chalcones, nocodazole (oncodazole) and taxanes (Rowinsky and Donehower, 1992).

Actin is the most abundant and highly conserved protein of eukaryotic cells, with 80% homologies shared amongst species ranging from amoeba to man. It has major contractile and structural roles in maintaining the cell form, and in motility of muscle and non-muscle cells

**Table 2.** Adhesion changes.

| ROUNDING MEDIATOR | CELL TYPE | REFERENCES |
|---|---|---|
| Fibronectin-integrin, and Arg-Gly-Asp (RGD) sequence modulation. | Endothelium, cell lines, BHK, Vero kidney cells, embryonal cells, melanoma. | Ihara et al., 1982; Dienes et al., 1985; Rieber et al., 1988; Ingber and Folkman, 1989; Hynes, 1992; Ruoslahti et al., 1992; Bay et al., 1993. |
| Modulation of vinculin focal adhesion phosphorylations via GTPase and protein kinase C pathways. | *Xenopus* XTC fibroblasts, endometrial stromal cells, transformed fibroblasts. | Nigg et al., 1986; Carter et al., 1992; Symons and Mitchison, 1992. |
| Anti-adhesion glycoproteins, viz. tenascin (syn. cytotactin, J1, hexabrachion and glioma-mesenchymal extracellular matrix antigen), SPARC, thrombospondin, lectins. | Cell lines, embryonic cells, aortic endothelial cells. | Knudsen et al., 1981; Ekblom and Aufderheide, 1989; Funk and Sage, 1991; Sage and Bornstein, 1991; Zanetta et al., 1994. |
| Cytochalasin D on proteoglycan synthesis, and syndecan expression. | Chondrocytes, developmental cells. | Newman and Watt, 1988; Bernfield and Sanderson, 1990. |
| Tumor promoters, and growth associated protein GAP-34. | Tongue epithelial cells, cell lines | Arenholt et al., 1987; Widmer and Caroni, 1993. |
| Proteolysis by collagenase, trypsin, clostripain, plasminogen activation, plasmin and thrombin-like protease activity. | Chondrocytes, HT-1080, kidney fibroblasts, BALB/3T3, NB2a/dl neuroblastoma cells. | Thyberg and Moskalewski, 1984; Tryggvason et al., 1987; Ciambrone and McKeown-Longo, 1990; Mueller et al., 1992; Shea and Beerman, 1992. |
| Scraping (with "rubber policeman"), mechanical vibration («vortexing») electrofusions, and divalent ion chelation. | Multiple cell types, mosquito cells, and *Euglena gracilis*. | Pharmacia Fine Chemicals, 1981; Vannini et al., 1988. |
| Anti-attachment substrates, viz., silicon- and teflon-coating, agar gel and methylcellulose substrates, | Fibroblasts, and endothelial cells | Sank et al., 1993. |
| Proteolytic degradation of extracellular matrix, either induced or spontaneous as in malignant expression. Regulated by tyrosine phosphorylation of membrane proteins. | Chondrocytes, HT-1080, kidney fibroblasts, BALB/3T3, NB2a/dl neuroblastoma cells, RSV-transformed chicken embryo fibroblasts. | Tryggvason et al., 1987; Ciambrone et al., 1990; Mueller et al., 1992; Shea and Beerman, 1992. |

alike. Mitosis-specific phosphorylation of the actin-associated caldesmon and spectrin proteins via the $p34^{cdc2}$/maturation-promoting factor complex has been cited as causing the «rounding up» characteristic of many animal cell types (Yamashiro and Matsumura, 1991; Fowler and Adam, 1992; Norbury and Nurse, 1992). The cdc2-protein kinase is a major substrate of protein tyrosine kinases (Draetta et al., 1988), the largest group of oncoproteins in multicellular organisms, and implicated with a key role in transmitting signals from the outside of the cell to specific targets within the cell (Rodrigues and Park, 1994). In transformed cells, tyrosine phosphorylation via epidermal growth factor stimulation causes cell rounding with cytoskeletal and focal adhesion disruptions (Fry et al., 1994). However, dissociated responses have been reported (Defize et al., 1986). Tyrosine phosphorylation of adhesion-associated proteins, eg. tensin, $pp125^{FAK}$ focal adhesion kinase and paxillin are also seen stabilizing adhesions, promoting cytoskeletal assembly and the «well-spread» flattened morphology (Romer et al., 1992; Hidelbrand et al., 1993; Tidball and Spencer, 1993). Abolition of tyrosyl phosphorylation by genistein, a protein tyrosine kinase (PTK) inhibitor that inhibits all members of the Src protooncogene family of PTKs (Uckun et al., 1995) cannot prevent rounding in cells subjected to alkalinization induced by simple ionic treatments, viz., alkali or sulphate (Taylor, 1962; Sit et al., 1992h, 1993b), even when there is total abolition of basal and agonist-stimulated DNA synthesis (Sit et al., 1991c). Spontaneous alkalinization from proton pumping by an inserted heterologous $H^+$-translocating ATPase, is seen as the causal signal for transformation with rounding characterized by highly refractile profiles (Perona and Serrano, 1988; Perona et al., 1990).

Intermediate filaments are seen as mechanical integrators of cellular space (Lazarides, 1980), and desmosomal junction anchoring filaments (Bogner et al., 1992). The vimentin intermediate filament network is a major structural protein of mesenchymal cells like fibroblasts. Its morphology appears dependent of microtubules whereby microtubule depolymerization causes collapse of the intermediate filament array (Aubin et al., 1980; Goldman, 1985).

Microtubules are long cylindrical fibres assembled from the protein tubulin which has GTP binding sites that are important for the regulation of microtubule polymerization. Depolymerization by mitotic and antitubulin poisons produces rounding, an effect that is routinely produced by colchicine or colcemid in karyotyping to achieve synchronization via metaphase arrest. Cytoplasmic pH is seen to have a profound effect on microtubule movements where activation of centrifugal (kinesin) and centripetal (dynein) micro-

tubular motors appears implicated. Alkalinization or recovery from acidification, is associated with cell movements, hyperruffling of the cell edge, rounding and membrane fusion activity where lysosomal vesicles merge into larger tubular forms and move with the microtubules inwards centripetally towards the nucleus. Acidification is associated with cessation of cell movement, cell edge quiescence, cell spreading, and membrane «fission» activity where lysosomal vesicles remain small and round, distributed with the microtubules towards the cell periphery, centrifugally (Heuser, 1989).

### Adhesion changes

Adhesion of anchorage-dependent cells to the extracellular matrix (ECM) appears to initiate a number processes that have a bearing on rounding of cells, viz., signal transduction, and cytoskeletal organization, elevation of intracellular pH, growth and modulation of apoptosis (Schwartz et al., 1991; Juliano and Haskill, 1993; Boudreau et al., 1995; Miyamoto et al., 1995). Adhesion or «sticky» molecules are grouped as substrate adhesion molecules (SAM) and cell adhesion molecules (CAMs).

3 classes of substrate adhesion molecules are recognised: (1) collagen and elastins structural proteins, and (2) fibronectin, fibrillin, and laminin functional proteins, interacting with integrin receptors. The third class of substrate adhesion molecules are (3) the proteoglycans subserving adhesion functions and binding functions for growth factors to ECM and the cell surface, via mainly their carbohydrate moiety, viz. the glycosaminoglycans (GAGs, formerly called mucopolysaccharides) of (a) ECM, viz. versican, aggrecan, perlecan, decorin and fibromodulin, and of (b) the cell membrane (viz. heparan sulphate, dermatan sulphate, keratan sulphate, syndecan) (Kjellen and Lindahl, 1991). Signal transductions via GTPase, protein kinase C, growth associated protein GAP-43, and tyrosine kinase pathways modulate these adhesion interactions which have been implicated in cell rounding. Table 2 shows examples of these rounding responses.

Cell adhesion molecules have 2 major groupings of interest here, viz. the integrins and cadherins. Integrins are heterodimeric cell receptors for extracellular matrix proteins that mediate cell binding to a large number of different matrix constituents including collagen, fibronectin, fibrinogen, and von-Willebrand factor, triggering increased tyrosine kinase activity, intracellular pH and gene expression. Integrin-fibronectin interactions modulating rounding up of cells are well documented (see Table 2), while integrin-ECM interactions that are not fibronectin- or collagen-dependent have been shown to suppress interleukin 1ß converting enzyme (ICE)-mediated apoptosis which is characterized by rounding (Boudreau et al., 1995). Interleukin 1ß is a pro-inflammatory cytokine. Cadherins are divided into desmosomal and non-desmosomal cadherins (Edelman and Crossin, 1991; Edelman, 1992; Franke et al., 1992). Desmosomal (maculae adherents) adhesion proteins are intermediate filament (IF)-anchoring and sensitive to monocarboxylic acids (Bogner et al., 1992). The non-desmosomal adherens junction (AJ) proteins are actin microfilament (MF)-anchoring, associated with APC (adenomatous polyposis coli) tumor suppressor protein functions (Peifer, 1993; Rubinfeld et al., 1993; Su et al., 1993). Adhesion molecules modulating anchorage, movement, migration and invasiveness of the metastatic phenotype in transformed cells have often been reported (Juliano, 1987; Mueller et al., 1992).

The degree of adhesiveness needed to hold a cell in place has recently been studied by atomic force microscopy (AFM) (Dammer et al., 1995) which demonstrates that the binding of just a single pair of proteoglycan adhesion molecules is sufficient to hold the weight of an astounding 1,600 cells in a multicellular organism under physiological conditions. Since the hold of just one pair of adhesion molecules is already exceeding the requirement to secure the whole cell by 1,600 times, the diverse array of adhesion interactions implicated in anchorage seems overly complex and excessive in view of the fact that spontaneous release is a cyclical event in M-phase of the cell cycle, and an imperative requirement for movement and rounding. Anchorage release has been perceived as the primary mechanism initiating rounding, such as by proteolytic digestion which is used routinely in cell cultures and inhibitable by taxol (Smalheiser, 1991), by scrapping with a «rubber policeman» or cell scraper, and by expressing the malignant phenotype (Tryggvason et al., 1987). Gross redundancy in stickiness implies that no particular adhesive interaction could have any claim to critical relevance in cell anchorage. It is not certain that anchorage detachment is necessarily a «downregulation» of these formidable and multiple adhesive interactions in the sense that the articulating surfaces between cell and substrate at the adhesion joints become less sticky so as to allow separation. The study of integrin-cytoskeletal interactions in fibroblast migration shows that the cell is not detached from the substrate via separation between cell and substrate at the sites of focal adhesions, but the parts bearing the focal contacts are «ripped» off from the main cell body and jettisoned as the cell moves forward (Regan and Horwitz, 1992; Schmidt et al., 1993). Anchorage detachment with cell movements is therefore mechanistically a «rip off», and not a modulation of stickiness. The stuck areas of the cell are pinched off from the cell without them being unstuck. Cytoskeleton participation in his «rip off» mechanism of anchorage detachment is suggested as a delinking with the cell membrane at the focal adhesions. The family of ezrin-radixin-moesin (ERM) integral membrane proteins are tightly associated with microvilli or spikes throughout the cell cycle, thereby providing membrane attachment sites for cytoskeletal actin filaments (Sato et al., 1993; Yonemura et al., 1993). However, their involvement in «rip off» detachment is not known.

Cell detachment and rounding produced by the classical method of scraping with a «rubber policeman» or cell scraper is in fact the consequence of «rip off» detachment, similar to the spontaneous «rip off» detachment of anchorage sites in a moving cell. Here the cell body is being pushed by the «rubber policeman» rather than being pulled by the forward moving cell. With «rip off» detachment by scraping, the cell not only becomes rounded but also gets loaded with even huge cell-impermeant macromolecules. The cytoplasmic processes that have their anchorage ripped off by scraping, recoil and fold over any extracellular material in its proximity, thereby producing large endocytic channels. Membrane fusion of the folding lips then effects internalization of the extracellular material. Loading with «rip off» detachment is also known as «scrape loading» (McNeil, 1989). A migrating cell that moves on with «rip off» detachment would therefore be trapping macromolecules via «rip off loading» throughout its journey, tucking in a little along the way.

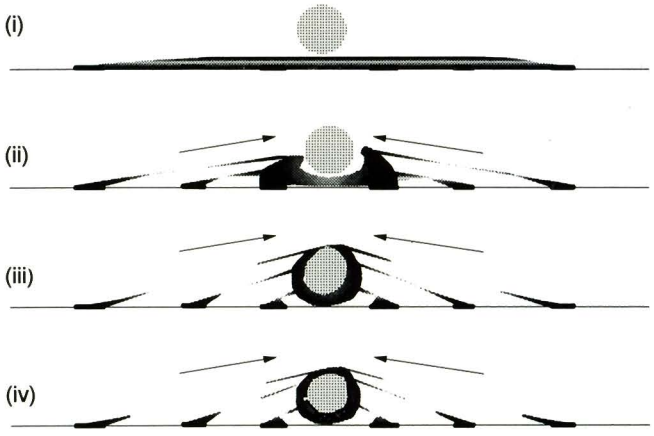



**Fig. 1.** Schematic illustration of cell rounding with concomitant large channel endocytosis and the «rip off» mechanism of detachment. The focal adhesion footplates are represented by dark dash lines on the substrate, under the cell in (i). In (ii) the cell is shown engulfing a large particle with «pinched out» anchoring microvilli connecting to the unyielding adhesion footplates. (iii) shows the cell having engulfed the big particle and is now rounded with some of the anchoring microvilli ripped apart. In (iv) the rounded cell is even smaller and show lysosomal processing of the endosome. Anchoring microvilli are ripped apart leaving behind on the substrate the adhesive footplates and parts of the ripped off microvilli.

## Reduced surface area

There is a long standing perception that membrane transport or flow modulates the overall shape and size of a cell (Van Deurs et al., 1989; Burger and Verkleij, 1990; Monck and Fernandez, 1992), as exemplified in the amoeba where «induced» endocytosis causes rounding with internalization of 50% of its cell plasma membrane surface within 30 minutes (Stockem and Wohlfarth-Bottermann, 1969). Rounding as a consequence of the plasma membrane invagination via large endocytic channels is illustrated in Fig. 1. The plasma membrane envelope becomes increasingly reduced in size by endocytic internalization, thereby causing the cell body to withdraw from the substrate. However what is also apparent is that the dense focal adhesion sites under the cell (Sit et al., 1991a-e) are not detached from the substrate with cell withdrawal, and that results in «pinched-out» actin-containing microvillus anchors or «guy ropes» connecting the withdrawing cell body from the focal adhesion sites (Fig. 1(ii), see also Sit et al.,

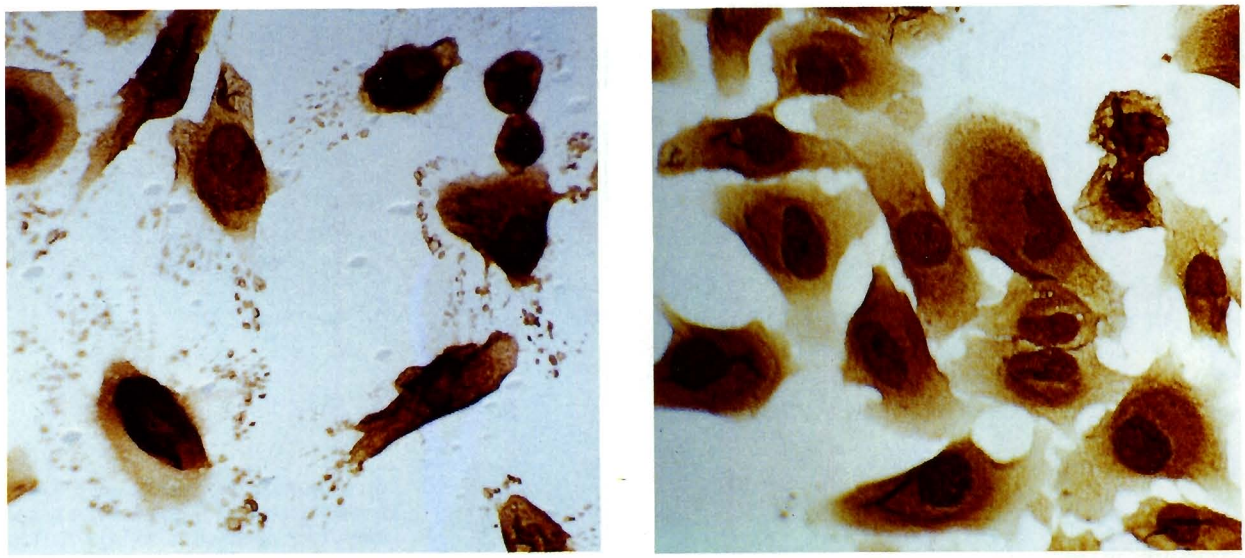
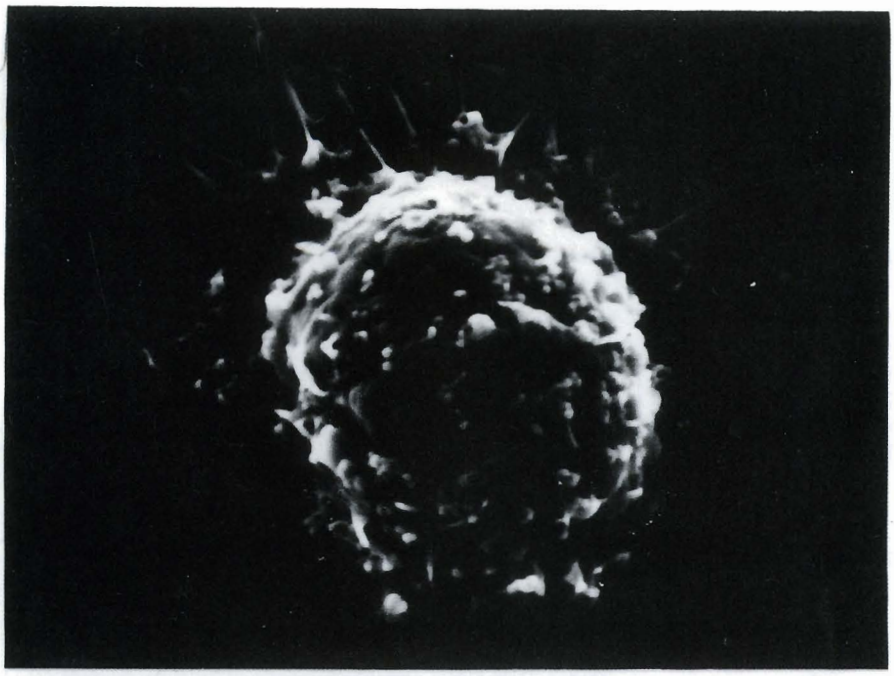


**Fig. 2. A** shows early stages of rounding in human Chang liver cells. The retracting cells leave behind a large number of jettisoned «body parts» which remain stuck to the substrate. This is the mutilating «rip off» detachment of rounding and is similar to the «rip off» detachment of a moving fibroblast (Regan and Horwitz, 1992; Schmidt et al., 1993). Rounding is induced by alkalinization in an acidification and recovery cycle using $NH_4Cl$ (Sit and Wong, 1989; Sit et al., 1994a). **B** shows controls without alkalinization. Neutral red staining (Sit et al., 1992a). Light microscopy. x 1,000



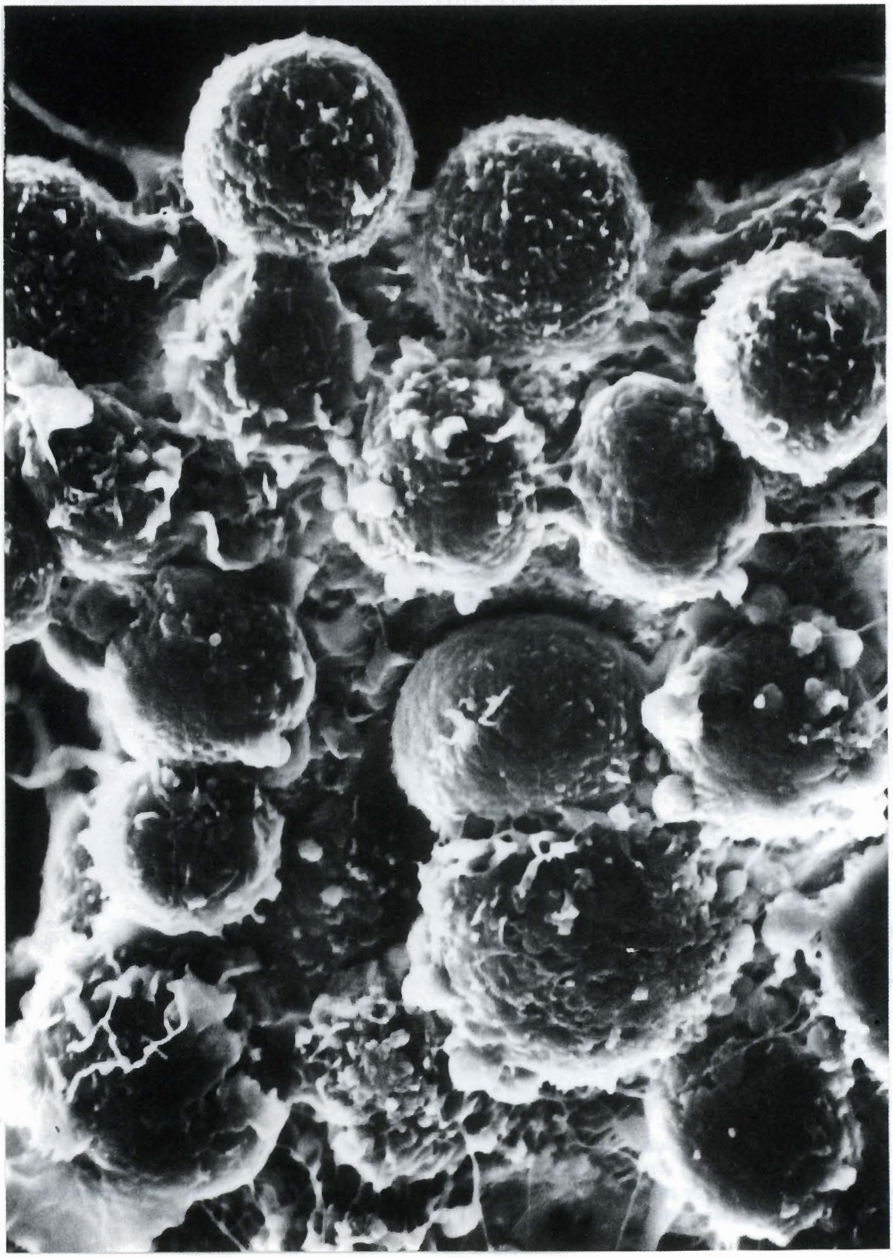


**Fig. 3. A.** Scanning microscopy of later stage rounding in a human Chang liver cell subjected to alkalinization as described in Fig. 2A. Ripped-off anchoring processes are surrounding the cell. **B.** Scanning microscopy of later stage rounding of human A431 epidermoid carcinoma cells incubated with 200 ng/ml epidermal growth factor (EGF). Rounded cells are lying over a mess of jettisoned «body parts». x 26,000

**Table 3.** Surface area reduction.

| ROUNDING MEDIATOR | CELL TYPE | REFERENCE |
|---|---|---|
| Mitosis, spontaneous. | Chang liver cells | Sit et al., 1992a, 1993a. |
| Apoptosis, from serum growth factor deprivation. | Chang liver cells. | Sit et al., 1994a |
| pH upshifting (alkalinization) via $Na^+/H^+$ antiporter exchanges, with and without epidermal growth factor enhancement; sensitive to saxitoxin, amiloride and quinidine. | Chang liver, human foetal liver | Taylor, 1962; Sit and Wong, 1989; Sit et al., 1991a,b,d, 1992b, 1993b. |
| Large channel endocytosis. | Amoeba, Chang liver cells, lung cells. | Stockem and Wohlfarth-Bottermann, 1969; Sit et al., 1990, 1991b, 1992c-e, 1994b; Bay et al., 1993. |
| Adenosine triphosphate (ATP) treatment. | Chang liver cells. | Sit et al., 1991c, 1992c, 1992f, 1992g. |
| Inorganic sulphate. | Chang liver cells, Cloudman malignant melanoma cells. | Sit and Wong, 1991; Sit et al., 1991b, 1992h; Bay and Sit, 1993. |

1992e). The withdrawing cell body in the meanwhile is becoming increasingly rounded because reduction in envelope volume would squeeze the cell mass and force it to assume a spherical form to maximize volume holding (Fig. 1(iii)). Continued traction rips apart the anchoring microvilli but not at the adhesion foot-plates which are left stuck to the substrate. The rounded cell is now free from any attachment and has an even smaller cell envelope. Compaction also gives the round cell a highly refractile appearance. Light microscopic profiles show clearly this mutilating «rip off» detachment mechanism in cell rounding (Fig. 2A). The substrate becomes littered with a large number of jettisoned «body parts» when cells «withdraw» from the substrate. Fig. 3A shows a scanning electron microscopic picture of a fully rounded cell surrounded by ripped-off anchoring processes. This mutilating detachment scheme is similar to the «rip-off.» detachment described in fibroblasts

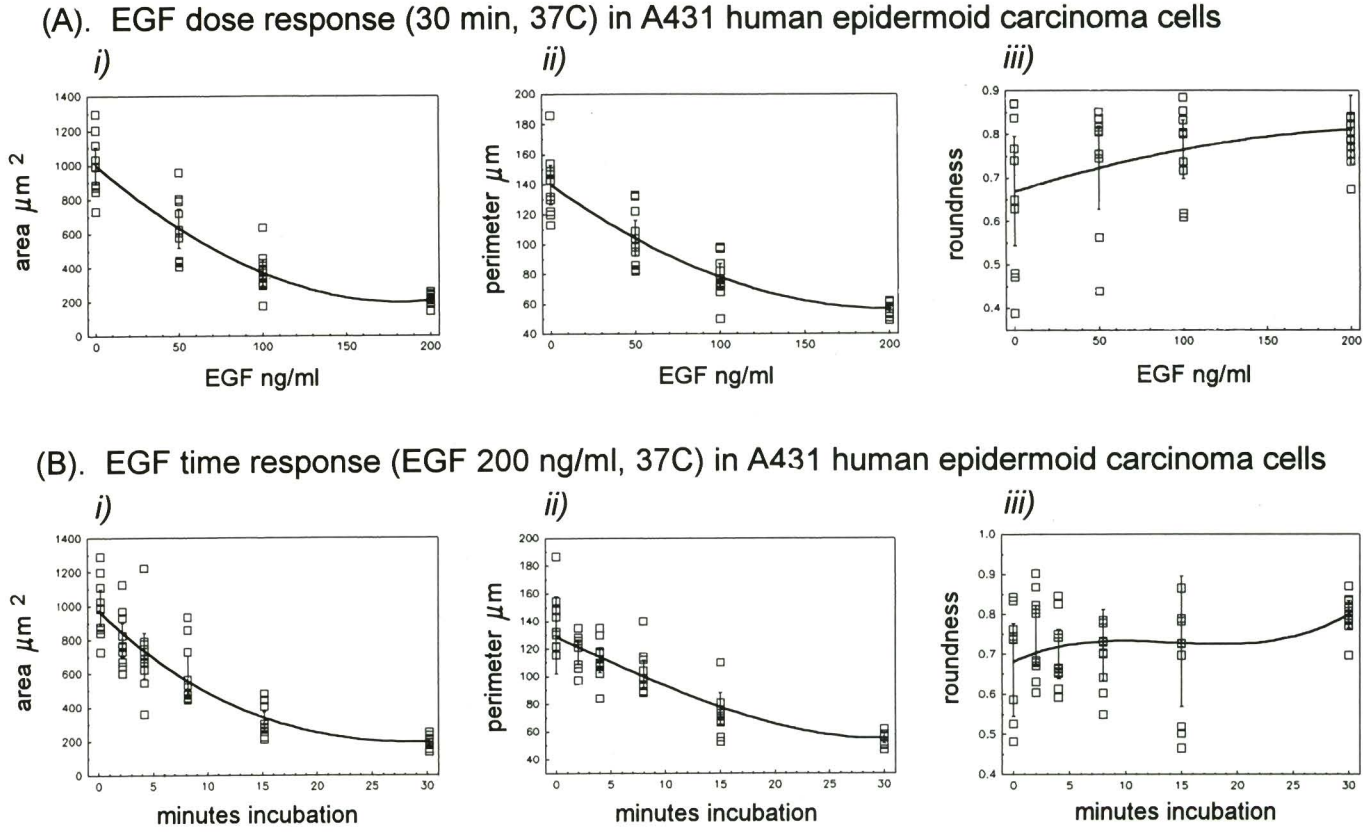



**Fig. 4. A.** Epidermal growth factor (EGF) dose response on A431 human epidermoid carcinoma cells. (i) profile area, (ii) profile perimeter, (iii) roundness form factor computed on-line in the Quantimet 570 image analyser:

$$PE = \frac{4 \cdot (\text{profile area})}{(\text{profile perimeter})^2}$$

(**B**) as in A except for being a time response.

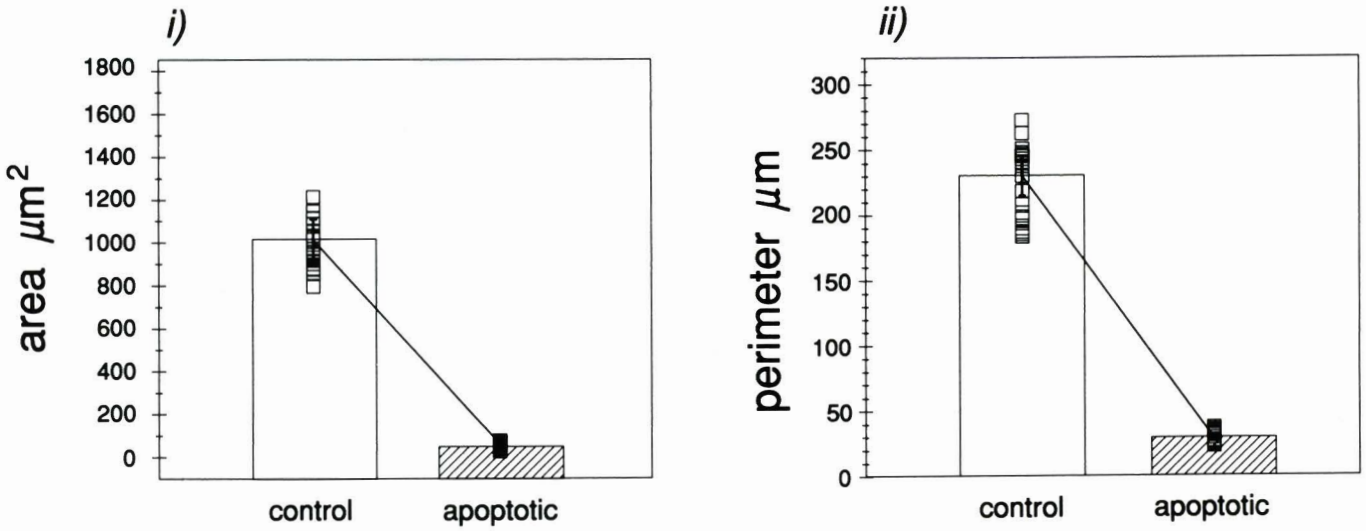



**Fig. 5.** (i) profile area of apoptotic human Chang liver cells from serum growth factor deprivation as described in Sit et al. (1994a), and of non-apoptotic counterparts. (ii) profile perimeter of apoptotic human Chang liver cells from serum growth factor deprivation, and of non-apoptotic counterparts.

moving foward (Regan and Horwitz, 1992; Schmidt et al., 1993). Even cells that are rounded by epidermal growth factor (EGF) treatment, such as in A431 human epidermoid carcinoma cells, leave ripped-off «body parts» behind on the substrate (Fig. 3B). EGF dose- (Fig. 4A) and time-responses (Fig. 4B) show significant profile area reductions with increase in the roundness form factor, suggesting a correlation between rounding and surface area reduction similar to that seen in interphase rounding induced by alkalinization, mitotic and apoptotic rounding (Table 3).

Surface area reducing activity that is concomitant with rounding is demonstrable in interphase human cells by intracellular pH upshifting via either directly imposed ionic gradients or signal transduction pathways, viz. with epidermal growth factor, ATP and sulphate (Table 3) that cause alkalinization (Sit et al., 1992e). This rounding up characteristic is not affected by the abolition of DNA synthesis and of tyrosyl phosphorylation by genistein which is a PTK inhibitor that inhibits all members of the Src protooncogene family of PTKs (Sit et al., 1993b; Uckun et al., 1995) but it is sensitive to $Na^+$-channel and $Na^+/H^+$ antiporter blockers, viz. saxitoxin, amiloride and quinidine (Sit et al., 1990, 1991b, 1992b,f, Table 3).

Internalization as the causation of reduction in surface area is demonstrated by the presence of massive endocytic activity (Table 3) via large endocytic channels and endosomes resembling the rounded amoeba (Sit et al., 1992d). Large 2 million mol.wt dextrans, latex beads of 1-2 μm diameter, heterologous chromosomes (mouse) and sperms (toad) (Sit et al., 1990, 1991b, 1992e, 1994b) are readily internalized with the induction of rounding by alkalinization. Surface area reduction together with large channel endocytic and membrane fusion activities are demonstrable in the early prophase to prometephase stages of mitotic rounding where alkalinization is a spontaneous and universal phenomenon (Sit et al., 1993a).

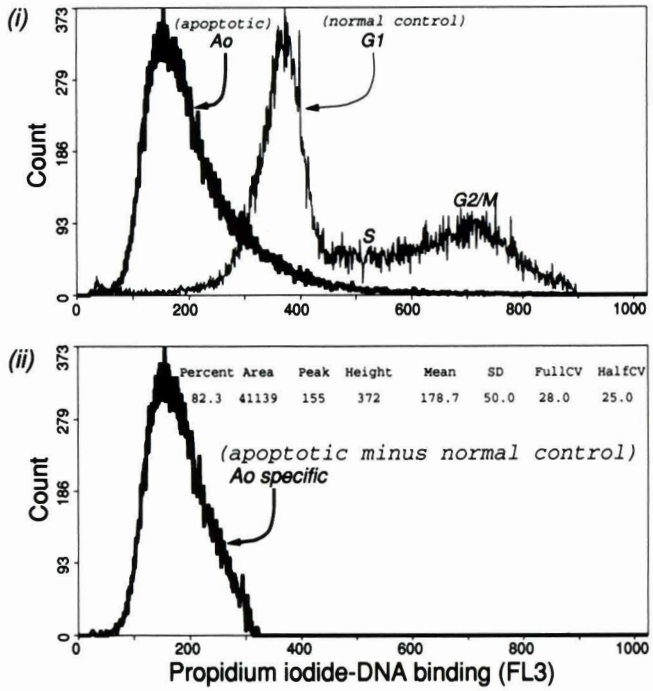



**Fig. 6.** (i) Cell cycle phase-specific DNA analysis of normal and apoptotic human Chang liver cells from serum growth factor deprivation. (ii) substraction profile showing apoptotic ($A_0$)-specific tracing representing almost total (82.3%) fragmentation of genomic DNA. Evaluated by the Coulter EPICS PROFILE II flow cytometer and EPICS ELITE Flow Cytometer Workstation programme, version 3.0.

Cells that are committed to undergo suicide in which they are said to die by apoptosis (programmed cell death), are also characterized by the «rounding up» change, complete with detachment, downsizing, raised density, and drastically reduced cell surface area (Fig. 5),

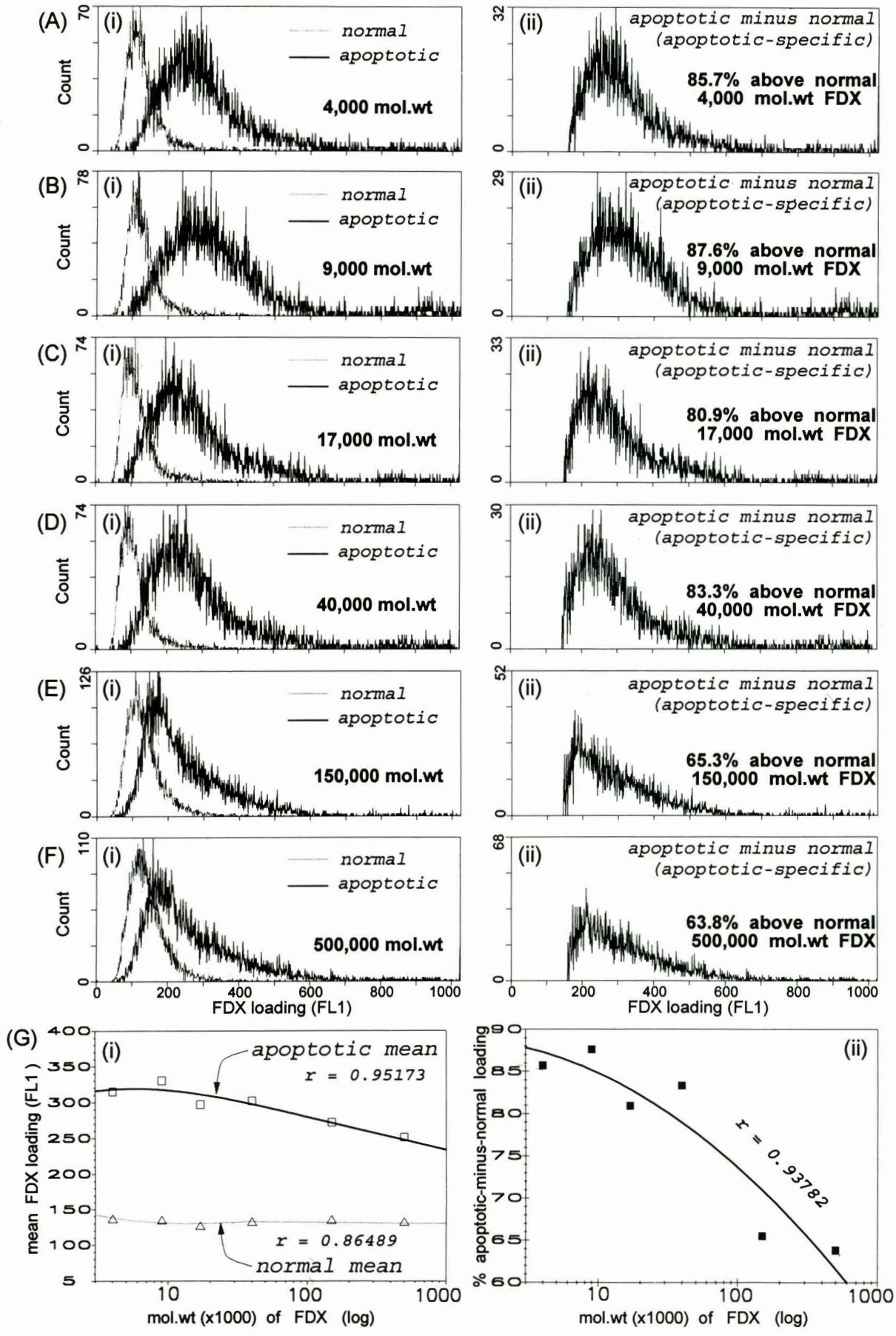



**Fig. 7.** Flow cytometric quantitation of fluoresceinated dextran (FDX, Sigma) uptake by apoptotic human Chang liver cells from serum growth factor deprivation. A(i) to F(i) are uptake histograms using FDX of 4,000 mol.wt. to 500,000 mol.wt., and their means plotted in G(i). A(ii) to F(ii) are corresponding subtraction histograms of apoptotic-specific uptake above normal, and their means plotted in G(ii).

just as in mitotic rounding (Sit et al., 1994a), except that here they are described as «cardinal morphological features of apoptosis» (Kerr and Harmon, 1991). However, unlike mitotic cells, the suicidal apoptotic cells have absolutely no proliferative capability, being distinguished by fragmentation of genomic DNA which is demonstrable in flow cytometry by the solitary $A_0$ apoptotic peak in cell cycle analysis (Fig. 6). The proliferative index ($S+G_2/M$) is zero, even $G_0/G_1$ DNA is destroyed. Apoptotic cells suffer from a «controlled autodigestion» that gives it a characteristic «moth-eaten» cytoplasmic appearance which is associated with intense acidification, thereby forcing a continual pH upshifting effort in attempted recovery (Sit et al., 1994a). Quantitation of fluoresceinated dextran (FDX) particle (4,000 to 500,000 mol.wt.) uptake by flow cytometry shows apoptotic cells from serum-growth factor deprivation, internalizing the huge FDX particles in a size-dependent manner, declining with increase in mol.wt. of the particles (Fig. 7A(ii) to 7F(ii) and Fig. 7G(i) to 7G(ii)). By contrast, normal cells have a baseline uptake pattern that does not change with change in size of the particles (Fig. 7A(i) to 7F(i) and Fig. 7G(i)). Uptake by apoptotic cells is very significantly higher (ANOVA $p<10^{-6}$). Massive endocytic internalizations would downsize the cells and contribute to the observed drastic reduction in cell surface area. Pervasive membrane fusion activity characterizes this apoptotic rounding phenomenon (see Sit et al., 1994a,b), supporting the perception that membrane fusion is of central importance here, as in mitotic and interphase rounding (Sit et al., 1990, 1993a) and in cell movements (Heuser, 1989).

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